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CYSTINE STORAGE DISEASE WITH AMINOACIDURIA AND DWARFISM

(LIGNAC–FANCONI DISEASE)

by

H. Bickel, H. S. Baar, R. Astley, A. A. Douglas,
E. Finch, H. Harris, C. C. Harvey, E. M. Hickmans, M. G. Philpott,
W. C. Smallwood, J. M. Smellie, and C. G. Teall

Almqvist & Wiksells Boktryckeri AB UPPSALA

FROM THE DEPARTMENT OF PAEDIATRICS AND CHILD HEALTH, UNIVERSITY OF BIRMINGHAM ; THE CHILDREN'S HOSPITAL, BIRMINGHAM ; THE DEPARTMENT OF CHILD HEALTH, UNIVERSITY OF SHEFFIELD ; THE CHILDREN'S HOSPITAL, SHEFFIELD, AND THE GALTON LABORATORY, UNIVERSITY COLLEGE, LONDON

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(LIGNAC-FANCONI DISEASE)

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To the memory of

SIR LEONARD PARSONS, F.R.S.

THIS WORK IS DEDICATED.

His interest in the work described in this supplement, his pioneer study of renal rickets, and above all, his friendship for the authors, we record with gratitude.

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PREFACE

This work on Lignac-Fanconi disease had its origin in Zürich, where during the years 1947-49 H. Bickel was Clinical and Research Assistant in Professor Fanconi's Department. In October 1949, by the good offices of the late Sir Leonard Parsons and Professor J. M. Smellie, Dr. Bickel joined the Staff of the Department of Paediatrics and Child Health in the University of Birmingham as a University Research Scholar, and the work of which this series of papers is a record has been carried out largely in the Birmingham Children's Hospital during the tenure of this scholarship.

The authors have been fortunate in having collected so much material in so short a time. To some extent this was due to the great generosity of Paediatricians and Pathologists throughout Great Britain and abroad, who have referred to us clinical cases, biological and full autopsy material for study within the Children's Hospital and have sent us abstracts of case histories of children under their care.

This work could not have been achieved without substantial help and co-operation from a large number of Medical Colleagues, Nurses and Technicians, both within the Birmingham Children's Hospital and outside, and it is not possible to mention by name all those to whom we are indebted. Wherever possible we have indicated in the text of this series of papers the source of the case or pathological specimen described. Our special thanks are due to : Professors G. Fanconi, J. Gough, F. Linneweh, A. V. Neale, D. S. Russell, A. P. Thomson, A. G. Watkins, Drs. H. Boehncke, R. J. K. Brown, W. T. Cooke, J. L. Emery, G. W. Hearn, H. Everley Jones, P. Gray, M. McGregor, H. Parry Williams, D. C. Thursby-Pelham, C. H. Snyder, W. Whitelaw and to our Clinical Colleagues in the Birmingham Children's Hospital who have so generously given access to their cases. We are grateful to Professor H. A. Krebs of the University of Sheffield, for numerous glutamine estimations and constructive criticism, to Dr. K. Schreier of the University of Heidelberg, for microbiological estimations, to Dr. C. E. Dent of the University of London, for his generous practical help and advice on chromatographic methods, to Drs. J. A. Barclay and

M. Ibrahim of the University of Birmingham, for many potassium estimations by flame photometer, to Dr. E. D. Lacey of the University of Birmingham, for the help in optical crystallography, and to Dr. R. W. H. Small also of the University of Birmingham, for X-ray crystallography studies. Thanks are also due to Dr. C. G. Parsons for his valuable criticisms and for the electrocardiography studies made on three of our patients, and to Dr. A. G. Marshall for the translation of the Dutch papers of Lignac. The loyal co-operation of Miss Mary Woods, Matron of the Birmingham Children's Hospital, and her hard-working staff has been invaluable, as was the assistance given by Miss Margaret MacCready, Mr. B. T. Rudd and other members of the Biochemical Department, and Mr. A. R. Detheridge, senior technician of the Pathology Department. The photography is the work of Mr. J. G. Williamson, medical photographer to the Hospital. The drawings in the ophthalmological section were kindly done by Mrs. Mary Young.

We are most grateful to the Endowment Research Fund of the Birmingham United Hospitals for grants used to provide technical assistance for the metabolic studies, and for defraying the whole cost of publishing this series of papers as a supplement. One of us (H.B.) is indebted to the University of Birmingham for a personal grant.

Cystine Storage Disease with Aminoaciduria and Dwarfism

(LIGNAC—FANCONI DISEASE)

PART 1 : INTRODUCTION

by

H. BICKEL, W. C. SMALLWOOD, J. M. SMELLIE, H. S. BAAR and E. M. HICKMANS

from

THE DEPARTMENT OF PAEDIATRICS AND CHILD HEALTH, UNIVERSITY OF BIRMINGHAM, AND THE CHILDREN'S HOSPITAL, BIRMINGHAM

History of the disease and general considerations

During the three years 1949-51 we have studied, in more or less detail, fourteen cases of cystine storage disease—a disorder which is generally considered to be an extremely rare one. In the families of these fourteen children seven siblings were found to have the disorder, making a total of twenty-one cases altogether. The seven have not been investigated in detail. In all but one of the fourteen children who have constituted the main material of the study cystine storage was demonstrated during life in the eyes and/or bone-marrow. General aminoaciduria, a condition which is explained later, was present in every one of the thirteen children whose urine was investigated. In view of these findings the name that best describes the disease is "cystine storage disease with aminoaciduria." As this term is somewhat unwieldy, we propose to use the names of the authors to whom we owe the first detailed descriptions of the condition and to call it "Lignac-Fanconi disease."

Of the fourteen children eight came from the Midland counties of England, and these include five from the Birmingham area. Six of these eight children have remained under observation in the Birmingham Children's Hospital for periods of from two months to more than two years.

The publication to which this is the introduction is based largely on the results of the study of these six children (Cases Nos. 1, 2, 4, 5, 8, 9) and on one other case (No. 3) observed previously by Professor FANCONI and one of us jointly. By the courtesy of colleagues in other medical centres in England we have seen four other cases, Nos. 6, 7, 10, 11 (two from the Midlands, Nos. 6 and 7) and have received from Germany and the U.S.A. data and specimens of three more (Nos. 12, 13, 14). These seven additional cases we have been permitted to publish either in full or, when the case is being published elsewhere, in abstract. Table 1 lists the patients, their place of origin, medical centre and other details.

Table 1.

Cases studied, places of origin and other details.

Case No.	Initials	Place of origin	Medical centre	Full publication by	Extent of study made by the authors
1	K.C.	Birmingham	Children's Hospital, Birmingham	The authors	Full clinical and biochemical study.
2	P.R.	Birmingham	Children's Hospital, Birmingham	The authors	Full clinical and biochemical study.
3	M.G.	Lausanne, Switzerland	Univ. Kinderspital, Zürich	Fanconi and Bickel, 1949	Full clinical and biochemical study by Fanconi and Bickel.
4	R.C.	Birmingham	Children's Hospital and Dudley Road Hospital, Birmingham	The authors	Clinical, biochemical and necropsy study partly by Drs. Hearn and Whitelaw, partly by the authors.
5	J.N.	Newcastle	Children's Hospital, Birmingham	The authors	Full clinical and biochemical study.
6	D.S.	Stoke-on-Trent	North Staffordshire Royal Infirmary, Stoke-on-Trent	Thursby-Pelham (planned)	Chromatography of urine and plasma. Examination of bone marrow. Visit to patient.

7	O.R.	Rugby	Children's Hospital, Bristol	The authors	Chromatography of urine. Examination of bone marrow. Visit to patient. Necropsy study performed by Dr. Lloyd. Histology by authors.
8	M.R.	Wolverhampton	Children's Hospital, Birmingham	The authors	Full clinical and biochemical study.
9	M.B.	Coventry	Children's Hospital, Birmingham	The authors	Full clinical, biochemical and necropsy study.
10 11	K.L. D.L.	Sheffield	Children's Hospital, Sheffield	Philpott et al. in this series of publications	Chromatography of urine and plasma. Visit to patients. Necropsy study of D.L. performed by Dr. Emery. Histology by authors.
12	A.M.	Marburg, Germany	Univ. Kinderklinik, Marburg	Linneweh, 1951	Chromatography of urine.
13	M.L.	Hamburg, Germany	Säuglingsheim, Hamburg	Boehncke et al., 1952	Chromatography of urine and plasma. Examination of bone marrow. Visit to patient.
14	J.S.	New Orleans, U.S.A.	Ochsner Clinic, New Orleans	Uncertain	Chromatography of urine. Examination of bone marrow.

The publication is divided into eight parts : 1. Introduction ; 2. Genetics of the disorder ; 3. General clinical description ; 4. X-ray changes in bones ; 5. Ophthalmological findings ; 6. Clinical description of Cases 10 and 11 by M. G. PHILPOTT, C. C. HARVEY and E. FINCH ; 7. Special biochemical aspects ; 8. Morbid anatomy, histology and pathogenesis. What follows immediately as part of this introduction includes a short review of the more important publications on Lignac-Fanconi disease, a general consideration of aminoaciduria, cystinuria and cystine storage, and finally a brief statement on the results of our own studies and the conclusions we have reached.

Rickets in association with kidney dysfunction was first described by LUCAS nearly 70 years ago. Since then the disorder has been reported so many times that in 1930 MITCHELL was able to refer to 76 examples of "renal rickets" in the literature. The excellent studies of MITCHELL (1930), HAMPERL and WALLIS (1933) and KAIYSER (1940), together with PARSONS' (1927) and TEALL'S (1928) classical descriptions of the bone changes, reveal "renal rickets" as a clinical and pathological condition which is both variable and complex and in our opinion is clearly without any single aetiology or pathogenesis.

In 1924 and 1926 LIGNAC described the results of his detailed study of 3 children who showed dwarfism, severe rickety deformities, albuminuria, glycosuria, polydipsia, polyuria, anorexia, constipation, vomiting and repeated attacks of fever. All succumbed to minor infection and in many tissues including the liver, spleen, lymph nodes, and kidneys, was found a crystalline substance which was identified as cystine. While some of this deposit was obvious on naked-eye examination, some was demonstrated only after careful microscopic investigation. Lignac noted the familial incidence of the disease, a sister of one of his patients being affected with it. He referred to the publications of ABDERHALDEN (1903) and KAUFMANN (1922), who had described, though not very fully, the first case of cystine storage disease. Ten years elapsed before RUSSELL and BARRIE (1936) published their observations on two further cases. We now recognise how keen was LIGNAC'S perception, for as long ago as 1924 he enunciated the basic problem of cystine storage, namely, whether the "excess of cystine" is due to impaired kidney excretion or to pre-renal disturbance of the protein metabolism. One feature of LIGNAC'S cases which he appears to have overlooked was their similarity to some examples of renal rickets.

In 1931 in his paper on "non-diabetic glycosuria in childhood" and during the years which followed, FANCONI studied a syndrome in young children which differed in several respects from classical renal rickets and to which the cases described by DE TONI in 1933 and by DEBRÉ in 1934 clearly belonged. This disorder was noted to be familial

but its pathogenesis was obscure, and in his account written in 1936 FANCONI chose the purely descriptive name "nephrotic glycosuric dwarfing with hypophosphataemic rickets in early childhood." Further contributions to the literature followed and were reviewed in the excellent paper of MCCUNE, MASON and CLARKE (1943). Publications not mentioned in that paper are those of van der ZIJL and HESLINGA (1940), van CREVELD and GRÜNBAUM (1941), and AIDIN and NOBEL (1942). Other communications since 1943 include those of HOTTINGER (1947), ULLRICH (1948), LINDER, BULL and GRAYCE (1949), HINGSTON (1949), D'AVIGNON and VAHLQUIST (1949), KING and LOCHRIDGE (1951), DRABLØS (1951), MONOD (1951), LINNEWEH (1951), SCHÜMMELFEDER (1952), DIMSON (1952), and two more papers by FANCONI in 1946 and FANCONI and BICKEL in 1949 (see Tab. 2, Part 8). These numerous publications have demonstrated that each individual feature of the disease described by FANCONI in 1936 shows a confusingly wide variability.

It has become evident that the disorder is not restricted to **early childhood.** RUSSELL's second case, verified by autopsy, lived to the age of 16 years, and the sister of LINDER's patient died of what appears to have been the same disease at the age of 22 years. Cystine storage was not demonstrated. **Kidney changes** were minimal in BENOIT's case (1935) and were absent in DRABLØS' (1951) and in HOTTINGER's case (1947), the youngest recorded in the literature. The variability in the degree of **glycosuria** was emphasised by FANCONI in 1936 and also the absence of clinical **rickets** in the three cases he described in 1946 and 1949. In the advanced stages of glomerular insufficiency **hypophosphataemia** may be replaced by **hyperphosphataemia,** though hyperphosphataemia is found sometimes even in the early stages and without any associated nitrogen retention (HOTTINGER 1941, LINDER et al. 1949). Indeed, from the earlier descriptions the one constant finding appears to be **dwarfing,** though this was but slight in PACHE's (1940) second case. The measurements of RUSSELL's (1936) second case were not recorded.

The difficulties in diagnosis which result from the great variability in the clinical manifestations of Fanconi's disease were clearly shown in MCCUNE's review in 1943. McCune divided the 39 probable cases described in the literature into 10 different groups, based on a combination of two or more cardinal symptoms, and concluded that the disease was "no sharply definable clinical entity." Since McCune published this conclusion, however, two other features have acquired increasing significance. They are **cystine storage** and **general aminoaciduria** and the association of these two findings together with dwarfing are of the greatest importance in establishing the diagnosis of Lignac-Fanconi disease.

A year after FANCONI's original publication in 1936, BEUMER and WEPLER drew attention to the close **similarity** between Fanconi's cases

and Lignac's cystine storage disease. This is true of all the seventeen cases of cystine storage disease in the literature which were reviewed by FREUDENBERG in 1949. All these cases fit the clinical description given by Fanconi of his disease. At the same time, if we include our own material, then the majority of the reported cases of Fanconi's syndrome have shown cystine storage.

That in some descriptions of Fanconi's disease cystine storage was not mentioned is not surprising and does nothing to disprove our contention that it is an essential feature of the condition. LIGNAC (1924) himself remarked that the stored cystine might be scanty, and had noted its rapid solubility post mortem in formalin, acid dyes, ammonia and even water. HOTTINGER (1941) drew attention to the fact that distinguished pathologists, including RÖSSLE (1938) and BENOIT (1935), had overlooked cystine storage or interpreted it wrongly. Our own experience is that cystine crystals in tissue are not always easy to demonstrate and that while a positive result is of the greatest importance, a negative result does not exclude cystine storage.

As to the diagnosis of cystine storage **in vivo,** suitable methods were first described in 1941 by BÜRKI and by ESSER, who identified cystine in cornea and conjunctiva by slit-lamp examination, and also in sections of conjunctiva and in bone-marrow removed at biopsy. However, cystine crystals in preparations of bone-marrow may be so scanty that they are found only after hours of search. They

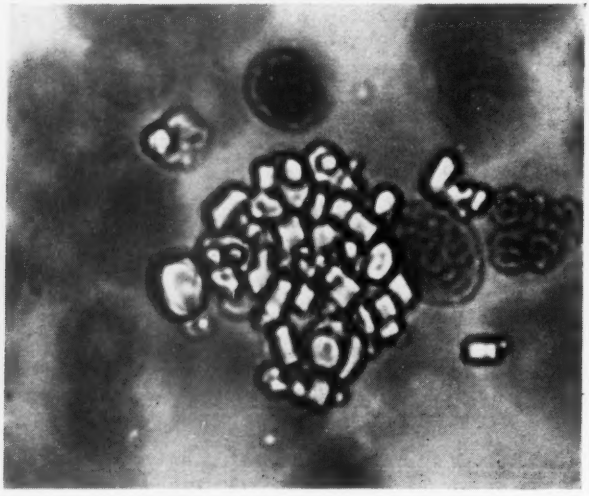


Fig. 1. Cystine crystals in the bone-marrow of one of our patients.

may dissolve in water or in the acid dyes used in preparing the slide for microscopy. The methods of microscopical, crystallographical and chemical identification of cystine in tissues will be discussed in Part 8. The use of the slit-lamp in demonstrating crystals of cystine in cornea and conjunctiva requires an experienced investigator, especially when the deposit is scanty and the child uncooperative. Fig. 1 shows a photograph of cystine crystals in the bone-marrow of one of our patients.

All but one of our cases of Lignac-Fanconi disease were investigated for **general aminoaciduria** and all were positive. By general aminoaciduria is meant the presence in the urine in fairly large amounts of 10 to 20 aminoacids. This contrasts with the finding of up to 6 aminoacids in cystine-lysinuria, phenylketonuria and some other disorders which are mentioned later. The aminoaciduria was first suspected in 1936 by FANCONI when he discovered a great increase of organic acids in the urine of his patients and suggested that they consisted in part at least of aminoacids. MCCUNE confirmed Fanconi's hypothesis when in 1943 he differentiated the organic acids present in the urine of his patient as 82 per cent aminoacid, 11 per cent lactic acid and 7 per cent beta-hydroxybutyric acid. Any closer quantitative or qualitative analysis of this aminoaciduria was delayed for many years because adequate methods of investigation were not available. Mild, variable aminoacidurias, such as are often found in Lignac-Fanconi disease, escaped notice, therefore, while the few aminoacids which were readily detected, in particular cystine, received undue emphasis.

Eventually a proper investigation of the aminoaciduria of Lignac-Fanconi disease was rendered possible by **paper chromatography**, a relatively simple and quite specific method of demonstrating individual aminoacids (CONSDEN, GORDON and MARTIN, 1944). DENT (1947) was the first to realise the clinical importance of this method in the investigation of disturbances of aminoacid metabolism. He used it in the study of an adult patient suffering from a disease similar to that described by Fanconi, and was able to demonstrate more than 18 different aminoacids in the urine of his patient. Stimulated by Dent's publication and aided by his invaluable personal advice, HERMANN, BICKEL and FANCONI adopted this new method and in 1949 applied it to the study of aminoaciduria in a child suffering from Lignac-Fanconi disease. In the same year LINDER and his colleagues and D'AVIGNON and VAHLQUIST used the method of paper chromatography for the same purpose.

Paper chromatography provides a simple and elegant method of detecting aminoacids in the urine and plasma of healthy children and of those who are suffering from various metabolic disorders. In the past two years we have carried

out about 5,000 such investigations and are convinced of the usefulness of the method. In particular, 500 urine and 150 blood specimens from children with Lignac-Fanconi disease have been tested for their aminoacid content. It was the discovery of aminoaciduria rather than the variable clinical picture which led us to look for cystine storage in these children.

With the growing importance of aminoaciduria as a diagnostic feature of Lignac-Fanconi disease, its **differentiation from other conditions associated with aminoaciduria** has become increasingly necessary (DENT 1949, BICKEL 1950, 1952, and Fig. 10, Part 3). The differences are readily demonstrated by chromatography (Fig. 2). Aminoaciduria is physiological in many newborn infants ; it is found in certain cases of liver disease and of steatorrhoea, in cystine-lysinuria, phenylketonuria, hepato-lenticular degeneration, galactosaemia and other conditions. A closer qualitative analysis of these aminoacidurias reveals that some have a characteristic aminoacid pattern which is quite different from that found in Lignac-Fanconi disease. Good examples of this are cystine-lysinuria and phenylketonuria. More general aminoaciduria with an increase in 10 or more aminoacids has been found in newborn infants (SOUCHON 1952, BICKEL 1952), in cases of hepatolenticular degeneration (DENT and HARRIS 1951, BICKEL 1952), in galactosaemia (HOLZEL, KOMROWER and WILSON 1952, BICKEL 1952), in association with severe liver damage (DENT and WALSHE 1951, BICKEL 1952) and also as the result of renal tubular damage of different types and aetiology. Thus, tubular damage with aminoaciduria has been described in vitamin D intoxication (van CREVELD 1949) and in glycogen storage disease (FANCONI and BICKEL 1949).

It is possible that the **syndrome described in adults** which is characterised by osteomalacia, glycosuria (HUNTER 1935, COOKE, BARCLAY et al. 1947) and aminoaciduria (DENT 1947, STOWERS and DENT 1947, MILNE, STANBURY and THOMSON 1952) is also due to tubular damage of unknown origin. This syndrome has been identified with Fanconi's disease of childhood. In the present state of our knowledge it is impossible to decide whether all conditions exhibiting the above symptoms are one and the same disease or are even interrelated.

Many authors, among them MCCUNE et al. (1943), and LINDER et al. (1949), have regarded **tubular insufficiency** as the essential underlying lesion in Fanconi's disease of childhood and have attributed to a low renal threshold the aminoaciduria, glycosuria and supposed phosphaturia. This attractive hypothesis can be traced back to FANCONI'S publication in 1936, in which he explained the glycosuria and acidosis as the result of faulty reabsorption in the tubules of sugar

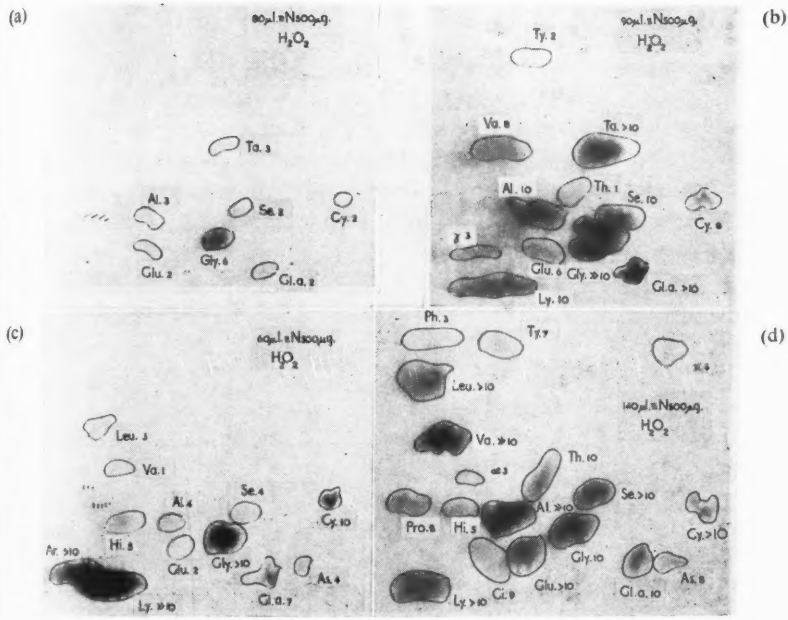


Fig. 2. Urine chromatograms of (a) healthy school child, (b) healthy newborn infant, (c) patient with cystine-lysinuria and (d) patient with Lignac-Fanconi disease. Al., alanine ; α, alpha-amino-n-butyric acid ; As., aspartic acid ; Ar., arginine ; Ci., citrulline ; Cy., cystine as cysteic acid ; γ, gamma-amino-butyric acid ; Gl.a., glutamic acid ; Glu., glutamine ; Gly., glycine ; Hi., histidine ; Leu., leucine + isoleucine ; Ly., lysine ; Ph., phenylalanine ; Pro., proline ; Se., serine ; Ta., taurine ; Th., threonine ; Ty., tyrosine ; Va., valine ; X, unknown substance. Figures at side of aminoacid names indicate relative colour intensities of spots—1 for weakest, $\gg$10 for strongest colour.

and bases. Now, however, tubular dysfunction is no longer acceptable as the whole explanation of the disorder—certainly not of the aminoaciduria and phosphaturia. The chemical studies of Professor Krebs and Dr. Schreier's microbiological estimations have shown that in several of our patients, and also in Philpott's cases, the level of various aminoacids in the plasma was raised. This is considered in detail in Parts 3, 6 and 7. The aminoaciduria appears, therefore, to be due to an overflow from the blood rather than to faulty reabsorption by the tubules, though tubular dysfunction as a contributing factor cannot be excluded until clearance work has been done in such cases. As for the

phosphaturia, none of the 10 metabolic balances recorded in the literature nor our own metabolic studies have revealed excessive phosphaturia, if the normal values given by MACY (1942) in her extensive balance studies are accepted as a basis for comparison. The phosphorus balances were usually negative in the absence of vitamin D medication, and this was due to abnormal loss of phosphorus in the stools. To a lesser degree this is true of calcium also (see Part 7).

The significance of cystinuria in Lignac-Fanconi disease. In 1903 ABDERHALDEN found cystinuria in the family of a patient with cystine storage disease and in 1926, at the autopsy of one patient, LIGNAC found cystine storage in the tissues and also cystine stones in the urinary tract. Eleven years later FREUDENBERG (quoted by PACHE, 1940) and FANCONI (1946) demonstrated cystine in the urine in this disease. Following this discovery, the cystinuria of Lignac-Fanconi disease was often identified with the classical benign form of cystinuria (cystine-lysinuria) first described by WOLLASTON in 1810. Variability in the clinical pictures presented by these two diseases was explained by the differences in age, while the basic disorder of the cystine metabolism was regarded as the same in each. This hypothesis is not supported by recent work on these disorders, using the method of paper chromatography, or by metabolic studies. The differences in the two may be considered under three headings.

1. **The extent of so-called cystinuria in Lignac-Fanconi disease.** In not one of the 500 urine chromatograms from cases of Lignac-Fanconi disease here presented did we find isolated or even predominant cystinuria. Indeed, the concentration of the other 10 to 20 aminoacids was generally stronger or at least as strong as that of the cystine, so that the use of the term "cystinuria" in connection with this disease is no more appropriate than "leucinuria" or "valinuria," and is undesirable because it directs attention to cystine alone. Examination of one urine specimen for cystine may be negative in Lignac-Fanconi disease, for the cystine excretion is variable and often remains normal despite a great increase in the excretion of other aminoacids. On the other hand, with the exception of phenylketonuria, urinary cystine may be found in any of the other disorders associated with aminoaciduria mentioned earlier. There are, indeed, at least 7 different forms of cystinuria between which the common test for cystine cannot distinguish and it follows that references in the literature to cystinuria as evidence of Lignac-Fanconi disease must be regarded critically.

2. **The relationship between the cystinuria of Lignac-Fanconi disease and classical cystinuria (cystine-lysinuria).** In contrast to the aminoaciduria of Lignac-Fanconi disease, classical cystinuria shows a constant and greatly increased excretion in the urine of cystine, lysine and often arginine as well. For this reason, the name "cystine-lysinuria" is more appropriate and descriptive than "cystinuria" and will be used throughout this publication. DENT and ROSE (1951) and BICKEL (1952) have each investigated more than 20 of these cystine-lysinurias. It is probable that the continuously high concentration of cystine in the urine in this form of cystinuria in particular leads to extensive cystine stone formation. A further explanation may be that patients with cystine-lysinuria generally excrete a urine of normal acidity whereas in Lignac-Fanconi disease the urine often has an abnormally high pH due to bicarbonate loss by the kidney. Secretion of an alkaline urine seems to prevent cystine stone formation (HICKMANS and SMALLWOOD, 1935 ; BICKEL 1952, observations in alkalinised patients with cystine-lysinuria) while neutral or weakly acid urine in cystine-lysinuria might well facilitate it. Nevertheless, there is no reason why other forms of cystinuria should not occasionally lead to stone formation, and in fact Baar has discovered microscopical cystine stones in the tubular lumen of two of our patients who died of Lignac-Fanconi disease and also in sections from Zollinger's case (see Part 8). Lignac's finding of a cystine stone probably has a similar explanation, so that cystine stone formation is not absolute proof of the existence of cystine-lysinuria. More work and the use of modern methods to differentiate more clearly between the various forms of cystinuria is required to establish whether or not intermediate states between cystine-lysinuria and Lignac-Fanconi disease occur. In our opinion there is no conclusive evidence of any transitional state. Not one of the children with Lignac-Fanconi disease studied by us showed the chromatographic pattern of cystine-lysinuria nor did patients with cystine-lysinuria show the clinical symptoms or profound metabolic upset of Lignac-Fanconi disease. Extensive investigation by chromatography into the families of children suffering from each disorder failed to reveal the occurrence of both diseases in the same family.* This is considered in more detail in Part 2.

*Recently, urine specimens of relatives of Case 4 have been tested by paper chromatography. The father, a paternal uncle and his son exhibited typical cystine-lysinuria without stone formation or cystine storage. We regard the association of cystine-lysinuria and Lignac-Fanconi disease in this family as a coincidence, as the urine of 138 relatives of other patients showed no cystine-lysinuria, and Lewis (1932) found the incidence of cystine-lysinuria in a healthy student population to be 1 in 600. Moreover, during our chromatographic investigations on the urine of 200 normal school children one child showed cystine-lysinuria, which shows that such a chance finding may well occur.

3. **The relationship between cystinuria and cystine storage.** In the past the generally accepted explanation of cystine storage has been that the infantile kidney is unable to excrete cystine either because of its immaturity (FREUDENBERG 1949) or because of toxic damage to the organ caused by cystine (LIGNAC 1924, BEUMER and WEPLER 1937) so that the cystine which cannot be excreted is stored in the reticulo-endothelial system. On this hypothesis the immediate cause of cystine storage would be the failure of the kidney to excrete cystine. This is not supported by our observations. Paper chromatography has shown that the infantile kidney is well able to excrete cystine, for investigation has revealed large quantities of cystine and other amino-acids in the urines of normal newborns in the first week of their life. Further, the large quantities of urinary cystine in cystine-lysinuria are excreted by the infant's kidney without causing glomerular insufficiency or cystine storage. Even among the relatively small number of cases of cystine-lysinuria in which stone formation caused hydronephrosis, anuria (the case of Dreadon et al. mentioned below) and finally kidney destruction with chronic uraemia (RUSSELL's third case 1936) there was no storage of cystine in the kidney or elsewhere.

Nine of the thirty cases of cystine-lysinuria studied by us have been children of whom three showed their first cystine stones in the second and third year of life, so that heavy cystinuria must have existed for a long time, probably from birth. We are indebted to Drs. Dreadon, Davison and Latner of Newcastle-on-Tyne for their account of a baby who at the age of eleven months had bilateral hydronephrosis and anuria caused by cystine stones in both ureters. The stones were removed and the child is now perfectly well, weighing 28 lbs. at two years of age. In all these six children, as well as the Newcastle case, bone-marrow puncture and slit-lamp investigation have failed to show evidence of cystine storage. Urea and phosphate values in the plasma were normal. The children were neither rickety nor dwarfed and their appearance was one of health.

Our studies on children with Lignac-Fanconi disease provide further evidence against a renal basis for cystine storage. Two of our patients (Cases 1 and 2) at quite an early stage showed cystine storage and abundant excretion of cystine in the urine but normal urea and low phosphate values in the plasma. Furthermore, HOTTINGER's (1947) and DRABLØS' (1951) description of two young cases of cystine storage are highly informative, for no kidney damage was demonstrated at the autopsy of either of them. This observation, together with the results of Baar's examination of post mortem and biopsy material from our cases (Part 8), have convinced us that cystine storage

in Lignac-Fanconi disease is the result neither of renal damage nor of cystinuria alone. We consider it to be the visible manifestation of a pre-renal disturbance of the whole aminoacid metabolism in which cystine, being poorly soluble, becomes deposited in the form of crystals, while the other aminoacids remain invisible in solution. Thus, whereas in the reticulo-endothelial system only the excess of cystine is apparent, in the plasma the level of many aminoacids is raised, as shown by chemical and microbiological estimations, while in the urine the full extent of the metabolic disorder is mirrored in general aminoaciduria.

Thus in the last 25 years a clearly defined clinical entity, namely, cystine storage disease with aminoaciduria or Lignac-Fanconi disease, has been isolated from the complex renal rickets group. The primarily extra-renal origin of this disorder appears to be established.

Summary

Fourteen children suffering from cystine storage disease with aminoaciduria (Lignac-Fanconi disease) have been studied in the last 3 years, and the results are described in a series of eight papers of which this first paper contains a short review of the literature, some general remarks on aminoaciduria, cystinuria and cystine storage, and a brief statement on some of the results obtained and conclusions reached.

Publications by Lignac, Fanconi, Beumer and Wepler, and others, as well as our own observations, lead us to the conclusion that Lignac's disease (cystine storage disease) and Fanconi's syndrome of childhood (nephrotic glycosuric dwarfism) are one and the same disease. At the present time this disease cannot be identified with similar syndromes in which cystine storage has been excluded.

The variability of nearly every symptom in Lignac-Fanconi disease is stressed. The most reliable diagnostic features besides dwarfing are cystine storage and aminoaciduria of a characteristic pattern. Cystine storage can be demonstrated in vivo by slit-lamp investigation in cornea and conjunctiva, as well as in bone marrow and lymph glands, and the cystine can be identified in biopsy material by microscopy, X-ray crystallography and chromatography.

The aminoaciduria is accompanied by aminoacidaemia, and the characteristic pattern in urine is best shown by paper chromatography. The differentiation from other forms of aminoaciduria, such as that of newborn infants and classical cystinuria, is discussed.

Reasons are given why cystine storage and aminoaciduria are regarded as the result not of kidney dysfunction but of a prerenal disturbance of the whole aminoacid metabolism, probably situated within the reticulo-endothelial system.

PART 2 : THE GENETICS OF LIGNAC-FANCONI DISEASE

by

H. BICKEL and **H. HARRIS**

from

THE DEPARTMENT OF PAEDIATRICS AND CHILD HEALTH, UNIVERSITY OF BIRMINGHAM, AND THE GALTON LABORATORY, UNIVERSITY COLLEGE, LONDON

1. The Familial Distribution

The occurrence of Lignac-Fanconi disease in more than one member of a family has been reported fairly frequently (LIGNAC 1924, FANCONI 1936, RUSSELL and BARRIE 1936, GITTLEMAN and PINCUS 1940, PACHE 1940, DANIS and ROSSEN 1941, van CREVELD and GRÜNBAUM 1941, ULLRICH 1948, FANCONI and BICKEL 1949, LINDER et al. 1949, HINGSTON 1949). The affected relatives were always brothers or sisters. In no instance has a family been described in which the disease occurred in more than one generation. Furthermore, the incidence of parental consanguinity appears to be raised. Marriages of cousins were recorded by DE TONI (1933), FANCONI (1936), PACHE (1940) and MCCUNE et al. (1943), whereas in many others there is insufficient information on the relationship, if any, between the parents. These facts suggest that the condition is genetically determined. However, the investigations of the families so far described are somewhat incomplete and no systematic investigation of the families of a series of cases of Lignac-Fanconi disease has yet been reported.

In the present investigation the relatives of eight cases of this disease have been studied ; seven of them belong to the group of patients dealt with in the other papers of this series, while one patient was investigated by one of us (H.H.) in collaboration with Dr. C. E. Dent at the University College Hospital, London. In each family a detailed clinical history of each relative was obtained and urine samples were collected from the majority. In all 138 urine specimens were examined for aminoacids by the technique of paper chromatography, and for reducing substances by the Benedict test and by paper chromatography. These findings may be summarised as follows :

(a) Sibs

Our eight patients had, in all, 19 brothers and sisters, but of these, only 12 were still alive at the time of the investigation. Among these 12, one was a typical example of Lignac-Fanconi disease. The other 11 were quite well and showed no gross abnormalities in urinary aminoacid excretion or glycosuria. Two of these sibs were still in their first year when we examined them and it is conceivable that

Table 1.

Details of the siblings of eight cases of Lignac-Fanconi disease.
(Sex and age at time of investigation or age at death is given for each individual).

Case No.	SIBS					Consanguinity of Parents	Miscarriages
	Original Case (Propositus)	Alive and definitely affected	Dead and probably affected	Alive and well	Dead : no evidence of affection		
1. K.C.	M. 1¼ Y.	—	—	M. 9/12 Y.	—	No	—
Harris and Dent	F. 1¼ Y.	—	—	—	—	Yes : 1st Cousins	—
2. P.R.	F. 1¾ Y.	—	—	M. 14 Y.	—	No	1
5. J.N.	M. 3 Y.	—	—	F. 6/12 Y.	—	No	1
6. D.S.	M. 2 Y.	—	—	—	—	No	—
8. M.R.	F. 6 Y.	—	M. 2½ Y. F. 7 Y.	F. 13 Y. M. 10 Y.	—	No	1
9. M.B.	F. 9 Y.	—	—	M. 18 Y.	M. 9/12 Y. F. 9 Y.	No	1
11. D.L.	M. 14 Y.	M. 7 Y.	F. 2½ Y. F. 6 Y.	F. 23 Y. F. 22 Y.	M. 3½ Y.	No	—

they may yet develop the disorder. Of the 7 sibs who were dead, 4 had probably died of Lignac-Fanconi disease. Two of these 4 children had severe rickets, did not walk and died of pneumonia. The other 2 were dwarfed and showed well-marked photophobia ; they died of "kidney disease." No investigations for urinary aminoacids or for cystine deposits in the tissues were made. The remaining 3 sibs had died at the ages of 9 months, $3\frac{1}{2}$ years and 9 years from meningitis, pneumonia and diphtheria respectively, and from the history of these cases there is no evidence to suggest that they had suffered from Lignac-Fanconi disease. The individual sibships are listed in Table 1.

(b) **Parents**

The parents of one of our patients were first cousins, the maternal grandmother and the paternal grandmother being sisters. No evidence of consanguinity was found in the other 7 families although this point was carefully investigated. All 16 parents were alive and well and in no instance was gross aminoaciduria detected. In one mother, lactosuria was detected during pregnancy, but no reducing substances were found in the urine of other parents.

(c) **Other relatives**

Among the other relatives there was no clinical history to suggest that any one of them had suffered from Lignac-Fanconi disease. Among the members of 3 families there was a history of diabetes mellitus in five. The grandfather of one patient had died of "kidney stones" that he had had from childhood. In all it was possible to examine the urines of 122 uncles, aunts, cousins and grandparents and in no instance was there any clear-cut aminoaciduria or excretion of reducing substances with the exception of one grandmother who suffered from diabetes mellitus, and whose reducing substance was identified by chromatography as glucose.

2. Mode of Inheritance

The familial distribution observed here is in general agreement with that recorded in the literature. The absence of any clear indication that the condition has occurred in more than one generation of the same family is against the hypothesis that the affected individuals are heterozygous for an "incompletely dominant" gene. Similarly, there is no evidence of sex-linkage. Both the present findings and the data in the literature are, however, consistent with the hypothesis that the disorder is inherited as a simple Mendelian recessive character. The occurrence of one affected and four probably affected sibs out of a total of 19 is in reasonable agreement with the 1 : 3 ratio expected theoretically, and the evidently increased incidence of parental consanguinity is strongly in favour of this interpretation.

It should be noted that in none of the relatives was there any case of the more common type of cystinuria in which there is an abnormal excretion of cystine, lysine and arginine but not of the other aminoacids (DENT and ROSE 1951, DENT and HARRIS 1951, BICKEL 1950,

1952).* This type of cystinuria is also inherited and runs true to type in individual families. In the past, Lignac-Fanconi disease might easily have been confused with cystinuria because in both conditions an abnormal excretion of cystine occurred in the urine. It now seems more likely that they are two quite distinct disorders with different genetical causations.

Among the relatives of our patients there is no evidence of the occurrence of the so-called Fanconi syndrome of adult life as described by MILKMAN (1930), HUNTER (1935), COOKE et al. (1947), STOWERS and DENT (1947), DENT and HARRIS (1951). The adult disorder resembles Lignac-Fanconi disease in showing a general aminoaciduria, hypophosphataemia, acidosis and glycosuria. It differs in that it does not develop until adult life when it presents itself as an osteomalacia. In none of these adults has cystine storage been demonstrated. In the present state of our knowledge we believe that the adult disorder is clinically and genetically distinct from Lignac-Fanconi disease of childhood.

2. **Incidence of Lignac-Fanconi disease**

An attempt has been made to obtain a rough estimate of the frequency of this condition in the general population. Five of our eight cases studied were drawn from the Wolverhampton, Coventry and Birmingham areas. In the course of the search for such cases, a considerable proportion of all known cases of resistant rickets, renal rickets and dwarfism of unknown aetiology in these areas had been examined and it is probable that the true total of living cases of Lignac-Fanconi disease in this area was not more than twice this figure. The total population of the Wolverhampton, Coventry and Birmingham county boroughs was estimated by the Registrar General in 1948 as 1,505,650. Of these, some 323,000 represent an estimate of the population under the age of 15. Thus, among living children under the age of 15, the frequency of Lignac-Fanconi disease was at least 1 in 65,000. This is probably an under-estimation of the true figure because it is unlikely that all living cases in these areas have been identified and also because there is a relatively high selective mortality against individuals with this condition, which inevitably leads to an incomplete estimate of their frequency in the sample. On the present evidence it is likely that the chance of any child developing this disease is not less than 1 in 65,000 and may be as high as 1 in 20,000.

If we take an incidence figure of 1 in 40,000 and accept the hypothesis that the affected individuals were homozygous for a single

*See footnote, Part 1, p. 19.

recessive gene, this would mean that the gene frequency was 1 in 200, and the frequency of heterozygotes, or "carriers", was approximately 1 in 100 of the general population. It is interesting to note that these estimates of gene frequency are very similar to those calculated by MUNRO (1947) for the recessive gene determining the condition of phenylketonuria.

4. Genetical Prognosis

The evidence in favour of the recessive inheritance of this condition is sufficiently strong to form a reasonable basis for genetical prognosis.

If two parents have already had one child with Lignac-Fanconi disease, the chances of any subsequent child suffering from the same disease are 1 in 4. The chances of any brother or sister of the parents having a child with this disease, while significantly higher than in the general population, are still extremely small, being probably about 1 in 800. If that brother or sister marries a first cousin then the chances of their having an affected child will be about 1 in 64. The chance that a brother or sister of a patient suffering from Lignac-Fanconi disease will have a child with the disease is about 1 in 600, unless they marry a first cousin when it will be as high as 1 in 48.

Summary

Lignac-Fanconi disease has often been described in siblings but never in different generations. Our findings in the families of eight patients, including chromatographic studies of 138 urine specimens, confirm these observations. Of a total of 19 siblings four were probably and one certainly affected. No other relatives suffered from the disease. Consanguinity was found in one family ; its incidence also seems to be raised in the literature. The data suggest that Lignac-Fanconi disease is genetically determined and of simple Mendelian recessive character, but that it is not genetically related to classical cystinuria or to the so-called Fanconi syndrome in adults. The frequency of Lignac-Fanconi disease in the general population is estimated to be roughly 1 in 40,000 with a gene frequency of 1 in 200. The genetical prognosis is believed to be 1 in 4 for any subsequent child of parents who already have one sick child. Corresponding figures are given for cousins of the patient and for children of the patient's sibs.

PART 3 : CLINICAL DESCRIPTION, FACTUAL ANALYSIS, PROGNOSIS AND TREATMENT OF LIGNAC-FANCONI DISEASE

by

H. BICKEL, W. C. SMALLWOOD, J. M. SMELLIE and E. M. HICKMANS

from

THE DEPARTMENT OF PAEDIATRICS AND CHILD HEALTH, UNIVERSITY OF BIRMINGHAM, AND THE CHILDREN'S HOSPITAL, BIRMINGHAM

A. CLINICAL DESCRIPTION

The clinical picture presented by Lignac-Fanconi disease in infancy and childhood varies considerably from case to case. There are wide differences in the age of onset, in the nature of the presenting symptoms and in the degree of constitutional disturbance. This wide variability in its clinical manifestations became evident in the first descriptions of the disease and is well illustrated in the fourteen cases upon which this series of communications is based.

The clinical manifestations are due to metabolic disturbances which affect the aminoacid and carbohydrate metabolism, the acid-base balance, the minerals potassium, calcium and phosphorus, the function of the kidneys and possibly of the reticulo-endothelial system. These disturbances result in dwarfing and wasting, rickets and osteoporosis, pyrexia, eye changes, anorexia, vomiting, polydipsia and polyuria, dehydration, acidosis, sometimes tetany, states of profound collapse and even sudden death. Laboratory investigations reveal amino-aciduria, glycosuria, sometimes albuminuria and ketonuria. In the plasma there is evidence of acidosis, hypopotassaemia, mineral changes as seen in rickets, and perhaps hypocalcaemia and uraemia, which may be renal, or extrarenal. A careful search reveals cystine crystals in the bone-marrow, conjunctiva and cornea.

Eight of our patients during their first two years of life showed signs of a severe widespread metabolic disturbance. The other six remained fairly well, requiring little or no medical care, until they reached school age, and showed as the only outstanding early clinical manifestations anorexia, dwarfing and, occasionally, bony deformity. For descriptive purposes the first group may be termed acute or subacute progressive infantile forms of Lignac-Fanconi disease, one in which death in metabolic crises tends to occur early. The second can be termed a chronic form, one which is consistent with survival till

puberty or possibly later, and one which usually terminates in uraemia secondary to kidney destruction or in an infection such as pneumonia or measles.

The acute and subacute forms of the disease

The symptoms generally commence some time after the age of six months, though an earlier onset has been observed by FANCONI (1936, Case 1, 1946, Case 2) and others. All the patients in our present series thrived and appeared in every respect normal during the first six months. The age of onset of symptoms is recorded in Table 1. Some of the earliest common manifestations are vomiting, refusal of food, abnormal thirst and constipation. As thirst increases, polyuria becomes a prominent feature, usually with incontinence ; all the acute and three of the chronic cases showed polyuria and polydipsia. Medical attention is often sought first because the child fails to grow or to gain weight, or actually loses weight.

The first medical examination usually shows the child to be undersized and more or less marasmic, with or without rickets (Fig. 1). Albuminuria, glycosuria, and pyuria are often present. The provisional diagnoses on reference or admission to hospital have included rickets, cysto-pyelitis, glycosuria, diabetes, gastro-enteritis and marasmus.

The age on admission is usually between one and two years. The most prominent feature is often dehydration, and the child's general condition may then resemble severe gastro-enteritis, though constipation is commoner than diarrhoea. Profound constitutional disturbance may be associated with high fever and this may have an obvious source in intercurrent infections to which young children with Lignac-Fanconi disease are especially susceptible. Often, however, the fever is associated with a normal blood picture and no apparent infection, and appears to have its origin in the metabolic upset itself. The pulse-rate is often high, the extremities cold, pale and cyanotic, and the breath indicative of ketosis. All solid food and even milk may be refused, but the child will drink water and fruit juices eagerly, often to vomit them immediately. He may drip urine almost continuously and demand drink hourly day and night. Starvation and repeated vomiting, by adding further to the dehydration, acidosis and hypopotassaemia, may be followed by circulatory collapse and a moribund state, from which recovery is possible only by prompt intravenous infusion therapy. The acidosis may be so severe that the CO_2—combining power of the plasma drops to 9 mEq/l or less, while the potassium may fall to 2.5 mEq/l, or even

Table 1

General clinical findings in 9 cases of Lignac-Fanconi disease.

	Case 1 K.C.	Case 2 P.R.	Case 3 M.G.	Case 4 R.C.	Case 5 J.N.	Case 6 D.S.	Case 7 O.R.	Case 8 M.R.	Case 9 M.B.
Age at onset of symptoms	6m	9m	12m	6m	9m	10m	15m	6m	1½ yr.
Age during observation	14m—2½y	1½—2¾y	1½y	1¼—3y*	2½—3y	1½—3y	2¼—2¾y*	6½—7y	9¾—10y*
Height below normal for age	—7 cm.	—6.5 cm.	—9 cm.	—10.7 cm.	—3 cm.	"dwarfed"	—5 cm.	—22 cm.	—31.5 cm.
Weight below normal for age	—0.7 kg.	—0.4 kg.	—1.5 kg.	—0.7 kg.	—3.3 kg.	—3.1 kg.	—1.8 kg.	—2.5 kg.	—1.3 kg.
Course	acute	acute	acute	acute	subacute	subacute	Chronic, early stage	chronic	chronic
Cystine crystals in eyes ..	+	+	—		+	+	—	+	+
Cystine crystals in bone-marrow ..	+	+	+	+	+	+	+	+	+
Temperature F° ..	96.5—102	97—104	98—105	97.5—104	97—101	97—101	96—102	97—101.5	96—103
B.S.R. mm. in 1 hr. (micro method) ..	15	10—17	23	24	12			10	

* died.

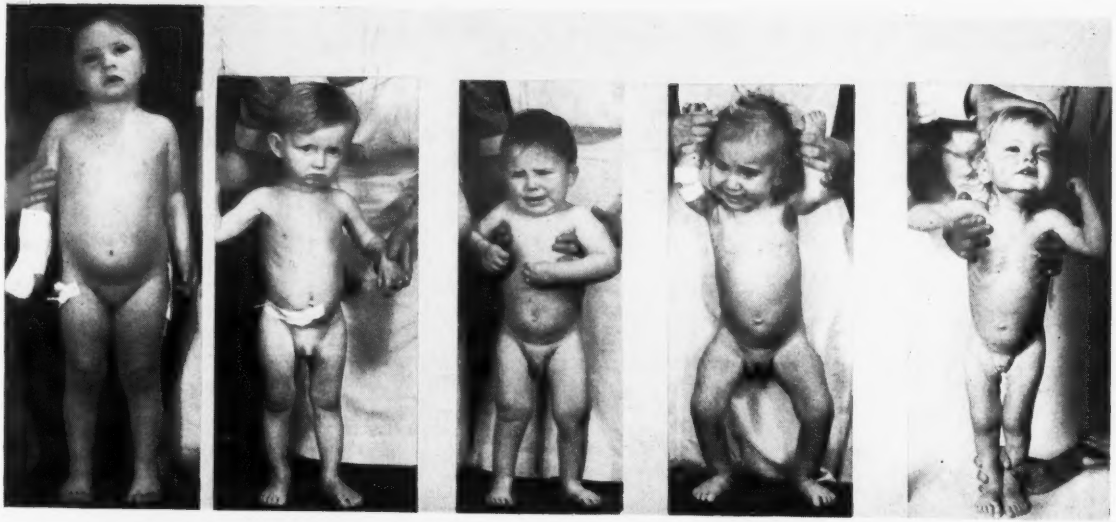


Fig. 1. Four children with the acute form of Lignac-Fanconi disease and a normal control.
From left to right : normal control, Cases 5, 1, 2 and 3.

lower. Unfortunately children whose general condition and appetite are reasonably good before admission to hospital tend to lapse into a metabolic crisis if unduly upset by change of surroundings or if the ward staff are ignorant of their great fluid requirement and finicky tastes. When one of our patients, despite all our efforts, continued to refuse food for several days after admission, we decided to send him home again, while three others received intravenous infusions during their first week in hospital.

The chronic form of the disease

The chronic form may present in various ways. In some the first symptoms may resemble those of the acute form but are much milder and commence later in life—generally towards the end of the second year or later. In others the disease presents as a problem in orthopaedics resembling resistant rickets and apparently unassociated with any serious upset of the general health which would suggest a renal or severe metabolic disorder. Others, in whom the presence of a kidney lesion has been recognised but its nature not fully appreciated, are likely to carry labels such as renal dwarfing, renal rickets or chronic glomerular nephritis.

The three most striking clinical features of the chronic form are dwarfing, bone changes which include rickets, osteoporosis and deformities, and photophobia. Dwarfing is the only constant finding. While this symptom has been mentioned as occurring in the young child with the acute form of the disease, it is a much more striking feature of the chronic form as it occurs in the older age groups (Fig. 2). The dwarfing is well balanced but the proportions tend to be those of a younger child, so that the appearance of trunk and limbs is in striking contrast to the much older facies.

The rickets, osteoporosis and bone deformities in no way differ from late simple vitamin D-deficiency rickets, except that the condition may have developed despite vitamin D prophylaxis and fails to heal with the usual therapeutic doses of the vitamin. As a result of lack of skeletal calcium some of the older children are confined to bed with bone pain, numerous pathological fractures and a variety of skeletal deformities. The commonest of the deformities is genu valgum, but genu varum, coxa vara, sabre tibia and bony changes in the shoulder girdle, thorax and spine are also seen (Fig. 3). The most severe X-ray changes (see Part 4) were observed in the oldest children in our series (Cases 9 and 11) who at the ages of 9 and 14 years were in a state of early chronic uraemia with hypocalcaemia and hyperphosphataemia. Tetany is not

Fig. 2. Chronic form of Lignac-Fanconi disease. Case 8, M.R., age 6½ years, compared with a healthy child (age 6¾ years).

Fig. 3. Early stage of the chronic form of Lignac - Fanconi disease combined with craniostenosis (Case 7, O.R., age 2¼ years). *Photograph by courtesy of Prof. A. V. Neale.*

uncommon in the chronic form of Lignac-Fanconi disease and is due to prolonged depletion of calcium in the bones and finally in the blood also. The fact that it does not occur more constantly is due to acidosis. Tetany may be precipitated by any hyper-ventilation, which may be associated with the emotional upset of admission to hospital, or by treatment with alkali. In contrast to these severely deformed and disabled patients it is remarkable that even in the later stages of the disease rickets may be almost or completely absent, so that a diagnosis of dwarfism or chronic glomerular nephritis is made (Case 8).

Photophobia and other eye changes are a common and often pronounced feature. Photophobia was present in half of our patients of all types, acute and chronic, and was a most striking abnormality in three of them (Fig. 2). One child brought his sun glasses into hospital, while another would play only when the curtains were drawn. Until now this symptom seems largely to have escaped observation, but when present it is of great diagnostic value, and its association with dwarfing

should suggest Lignac-Fanconi disease. Although some of our patients had unusually fair and scanty hair, photophobia was not limited to this group.

The photophobia led us to search for cystine deposits in cornea and conjunctiva (see Part 5), though later it became evident that these deposits are not always associated with photophobia. When the deposits are large, a hand lens will reveal haziness of the cornea, especially if a beam of light is directed obliquely through it. When the deposits are scanty, a slit-lamp is necessary for their demonstration and this investigation has presented no great difficulty, even in the youngest child, after proper sedation. Slit-lamp examination revealed cystine storage in ten out of twelve of our cases. Proof that the crystals were cystine was provided by X-ray crystallography (see Part 5, Fig. 6).

In the chronic form of the disease, especially, liver and spleen may be somewhat enlarged, findings which are explained by the histological changes caused by cystine storage in those organs (see Part 8). Uraemia may supervene in the last stages with manifestations such as diarrhoea, epistaxis, skin and intestinal haemorrhages, severe anaemia, high blood pressure, retinal exudate and haemorrhage, increasing drowsiness and anuria. In only two cases in this series (Case 9, age 9, and Case 11, age 14) could the early stages of chronic uraemia be observed, and in both patients infections supervened to cause death. The development of chronic uraemia with nitrogen retention and kidney insufficiency, similar to uraemia in chronic glomerular nephritis, is limited to a few cases and follows years of illness. It should not be confused with small increases in the blood urea, which are seen in early stages, are still reversible and are not, in our opinion, the result of glomerular insufficiency.

In the urine of the chronic cases traces of albumen are often, but not invariably, present. Sugar and ketone bodies are rarely found and then only in traces. Aminoaciduria is likewise far less striking than in the acute form. Microscopical examination may reveal a few red cells, pus cells, and hyaline or granular casts.

B. FACTUAL ANALYSIS AND DISCUSSION OF THE FINDINGS

There follows an account of findings that are of importance to the clinician in diagnosing and treating Lignac-Fanconi disease. A more detailed consideration of the aminoacid, sugar, electrolyte and calcium-phosphorus metabolism is given in Part 7.

1. General clinical findings

Of the fourteen patients of this series, nine were boys and five were girls. At the time they were under observation the eldest was fourteen years old, the youngest thirteen months, while nine were between one and three years. When symptoms first developed, eight were aged between six and twelve months, the rest were under two years.

Dwarfing was present in all and was most striking in the older children. Among children of the same age the degree of dwarfing varied with the severity of the disorder. For instance, Case 4, with an acute and severe form of the disease, was 10.7 cm. below average height at the age of 15 months, and died eighteen months later. Case 5, with a milder form, was only 3 cm. below average at the age of 2 years 6 months. Severely ill children may stop growing altogether ; Case 2 grew less than 1 cm. between the ages of 18 months and 2 years 9 months. This dwarfing is probably directly related to the profoundly disturbed protein metabolism. All are **under-weight** even when compared with a normal child of the same height. The more stunted the child, however, the less the proportionate weight reduction. Emaciation is seldom as striking as in most examples of the coeliac disorder or pancreatic fibrosis, and it seems probable that in Lignac-Fanconi disease fat and carbohydrates are sufficiently well absorbed and utilised to avoid great emaciation so long as growth remains stunted. The weight charts commonly show erratic variations which reflect the alternating dehydration and mild oedema which so often occur in the acute form of the disease. Periods of **fever** and raised blood **sedimentation rate** are also common features, especially in the acute form. They are often attributed to the greater liability to infection that these children undoubtedly show, though fever also occurs without obvious infection, and may then be interpreted as "thirst fever" (FANCONI 1936) or as a brain stem reaction to circulating products of the disturbed aminoacid metabolism. Similarly, the raised sedimentation rate is not always the result of intercurrent infection. Decrease in packed cell volume due to anaemia accelerates the sedimentation rate considerably. In Cases 2 and 8

Dr. HARDWICKE, of the Department of Experimental Pathology, Birmingham University, found a haematocrit value of 22 and 30 per cent (normal 38 to 40 per cent), in itself a decrease sufficient to raise Wintrobe's sedimentation rate to 30 mm./1 hr. A rise in sedimentation rate may also be caused by an increase in plasma fibrinogen or α_2-globulin, one or both of which have been observed in certain of our patients (see later).

Haematological investigation of peripheral blood and bone-marrow showed no characteristic or constant change. Normochromic anaemia was present at times in several of our patients, but was generally mild, and improved under general therapy with alkalis, etc. (see page 75). Leucocytosis with neutrophilia or lymphocytosis was found from time to time in most patients. There was no eosinophilia or monocytosis or other abnormality of the white-cell picture (see Table 2).

Cystine storage was demonstrated in all patients in this series, usually in bone-marrow, cornea and conjunctiva. The method of detection used is described elsewhere (Parts 5 and 8). Fig. 1, Part 1, shows a conglomeration of cystine crystals in the bone-marrow of Case 5. Crystals found in the conjunctiva were identified as cystine by X-ray crystallography (see Part 5). The high cystine content of bone-marrow samples was demonstrated by paper chromatography. Using this method a strong cystine spot was found in chromatograms made from 0.2 ml. bone-marrow punctate previously deproteinised and desalted. Little or no cystine was recovered from a similar volume of peripheral blood from the same patient or from the bone-marrow of patients who were not suffering from the disease. The general clinical findings in nine of our patients are summarised in Table 1.

2. **Rickets and calcium-phosphorus metabolism**

Of the fourteen patients eleven were rickety. Three showed no rickets, and of these only one had received more than the usual prophylactic dose of cod liver oil. This observation does not support MCCUNE's (1943) statement that rickets is an essential feature of the disease. The rickety bone changes are most marked in the older children. In their radiological appearance they do not differ from simple avitaminosis-D rickets of infancy and young children. This opinion is not shared by all workers, and a detailed discussion of the X-ray findings is given in Part 4. Unlike most renal rickets, in which hyperphosphataemia is the rule, rickets in Lignac-Fanconi disease is normally associated with pronounced hypophosphataemia and this is generally believed to be due to loss of phosphorus through the kidney. However, a survey of all

Table 2.

Haematological findings in 9 patients with Lignac-Fanconi disease.

	Normal	Case 1 K.C.	Case 2 P.R.	Case 3 M.G.	Case 4 R.C.	Case 5 J.N.	Case 6 D.S.	Case 7 O.R.	Case 8 M.R.	Case 9 M.B.
Hb. grams % ..	11.8—14.7	10.3—14.7	7.3—14.0	9.0—11.6	11.0	8.8—14.0	10.5—14.1	12.4—14.6	10.3—11.0	6.6—11.0
R.B.C. mill./mm.^{3} ..	4.5—5.5	4.4—5.0	2.9—5.2	3.6—4.4	3.5	3.4—5.0	5.6	4.3	4.5	3.0—3.3
Colour Index ..	0.9—1.1	0.7—0.9	0.9—1.1	0.8—0.9	0.9	0.8—0.9	0.9	1.0	0.8	0.8—1.0
Reticulocytes % ..	0.5—1.0	0.1—1.0	0.2	0.9					1.6	1.5—0
W.B.C./mm.^{3} ..	6—13,000	9—14,000	6—24,000	9,000	17—39,000	6—12,000	15—37,000	9—12,000	7,000	9—42,000
Differential blood count	Normal	Normal, neutrophilia or lymphocytosis	Normal, neutrophilia or lymphocytosis	Normal or neutrophilia	Neutrophilia or lymphocytosis	Normal	Normal or lymphocytosis	Normal or neutrophilia	Normal	Normal or neutrophilia
Platelets 1000/mm.^{3}	250—450	300	280	470					90	
Prothrombin time by modified McPherson technique	18—25″	25″	21—27″						27—34″	29″
Bone-marrow* ..		Erythropoesis and leucopoesis normal.	Low erythropoetic activity, otherwise normal.	Reduced erythropoesis. Some erythroblasts in caryorrhexis. Neutrophilia of 66.0%		Low erythropoetic activity. Small lymphoid cells near the upper limit of normal.			Normal erythropoesis, slight increase in reticulum cells. Leucopoesis normal.	Low erythropoetic activity, slight increase in reticulum cells. Myeloid series normal.

* For details see Part 8.

calcium and phosphorus balances previously recorded, together with the results we have obtained in the present series of cases, demonstrates that increased urinary excretion of calcium and phosphorus does not occur except under treatment with massive doses of vitamin D (see tables of balances, Part 7). Negative balances are all shown to be due not to loss of the minerals in the urine but to faulty absorption from the intestine. The balance experiments thus resemble balances in ordinary and resistant rickets. Rickets in Lignac-Fanconi disease does not respond to ordinary dosage of D vitamin but is healed eventually by giving massive doses of between 100,000 and 300,000 units daily for some weeks. That adequate absorption of phosphorus and calcium is achieved in this way is demonstrated in the admirable metabolic studies of GITTLEMAN and PINCUS (1940), which were continued over a period of several months, and showed a change from a negative to a positive balance under vitamin D therapy. In our series Case 1 also showed a positive balance after high doses of vitamin D. Gradual cure of the rickets is shown in the X-ray photographs in Part 4. A normal level of the serum phosphorus is not achieved until the skeleton is replete with minerals. Reversion to low values occurs when the massive vitamin therapy is ended and absorption of phosphorus from the intestine once more becomes deficient.

It might be argued that the absence of rickets in three of our patients could be explained by their stunted growth. One of the three (Case 5), however, was but little below normal height, which suggests that the extent of the bone changes is governed by variations not only in growth but also in intestinal mineral absorption. When absorption has been defective for some years severe bone changes develop, despite minimal growth, and there occurs generalised osteoporosis with multiple fractures and bending as in Case 9. In less severely ill children who continue to grow fairly well typical rickety changes at the metaphyses will occur.

Although hypophosphataemia is usually found in Lignac-Fanconi disease, normal and high serum phosphorus values are reported in the literature and have been observed in some of our cases. One of the fourteen children in our series showed a high plasma phosphorus level in every estimation, three others occasionally, while five sometimes had normal values, all without vitamin D medication. Still more remarkable was the fall in the usually normal plasma calcium level to values as low as 6 mg. per cent and less in four patients.

These two curious features of the chemistry of Lignac-Fanconi disease are well known from the publications of DEBRÉ (1934) and

HOTTINGER (1941). Hyperphosphataemia is generally interpreted as evidence of terminal glomerular insufficiency, while hypocalcaemia, as in classical renal rickets, is attributed to hyperphosphataemia. Many of our own observations cannot be interpreted in this way. Hyperphosphataemia is not always associated with uraemia, as would be expected in glomerular insufficiency. Further, it is often an early and completely reversible change, unlike the changes in blood chemistry in advanced chronic glomerulo-nephritis, in which hyperphosphataemia is a late and irreversible occurrence of ill omen. Hypocalcaemia may be accompanied by normal phosphorus levels, and cannot, therefore, be the result of hyperphosphataemia. In Case 9, serum calcium values of as low as 5.7 mg. per cent were recorded simultaneously with normal phosphorus values of 4.8 mg. per cent. When low serum calcium levels are raised by administration of calcium, high phosphorus values return to their original low level. Intravenous calcium therapy in Case 9 resulted in a rise in the plasma calcium level from 6.8 to 9 and later still to 12 mg. per cent, while the phosphorus level fell from 8.4 to below 1 mg. per cent.

Hyperphosphataemia and hypocalcaemia in Lignac-Fanconi disease must, therefore, be attributed to some mechanism other than that suggested for the uraemic stage of classic renal rickets or chronic glomerulo-nephritis.

We put forward the following explanation :

(a) **The hypocalcaemia** is the eventual result of years of calcium and phosphorus deprivation due to faulty mineral absorption. That the hypocalcaemia usually appears long after the development of hypophosphataemia is probably due to a more efficient regulating mechanism, which maintains a normal level of calcium in the blood as long as possible. This appears to be true also of simple vitamin D deficiency rickets. The eventual drop in the serum calcium level occurs when either a relative failure of the parathyroid to deal with the increasing demand of maintaining a normal calcium plasma level develops or when the skeleton is progressively deprived of the available calcium stores (see Part 8). The first explanation has been offered by LINDER, BULL and GRAYCE (1949) for the temporary low calcium and high phosphorus in the blood of their patient. Only in the terminal stages will hyperphosphataemia due to glomerular insufficiency cause further depression of the serum calcium level.

(b) **Hyperphosphataemia,** when it develops without renal failure, does so as a result of the low calcium level in the plasma, which brings to an end the deposition of calcium phosphate in the bone. This leads to an accumulation in the blood-stream of the phosphorus absorbed, a condition which may be reversed when the calcium level returns to normal. Hyperphosphataemia in Lignac-Fanconi disease is usually less pronounced than that of renal rickets. As in renal rickets, abnormally high levels are to be expected only in the very last stage, when the renal phosphorus clearance has become grossly defective.

Table 3.

Phosphorus and calcium findings in plasma and urine of 9 patients with Lignac-Fanconi disease.

	Normal	Case 1 K.C.	Case 2 P.R.	Case 3 M.G.	Case 4 R.C.	Case 5 J.N.	Case 6 D.S.	Case 7 O.R.	Case 8 M.R.	Case 9 M.B.
Rickets	—	+++	++	—	—	—	++	+	+	++
Plasma Phosphorus mg.%	4—5.5	2.1⇄4.8	1.3⇄5.5	1.9—2.5	6.2—6.5	2.0⇄5.5	2.0⇄5.2	1.5—2.9	1.9⇄6.1	0.8*⇄8.4
Plasma Calcium mg.% ..	9—11	10.8—11.8	9.3—11.5	9.2—11	10.7	7.8—10.5	10.0—10.5	9.5—10.5	9.9—11.8	5.5—12.4*
Alkaline phosphatase units (Kay)	8—12	24—41	8—15	4—6	20	13—19	36.6—50	25—45	10—13	16—28
Urinary Phosphorus mg/kg/24 hrs.	25—35		27.6	32		24.8	22.9	25.8		
Urinary Phosphorus % total excretion ..	60—68%	61%*							50%	51%
Urinary Calcium mg/kg/24 hrs.	3—6		1.2			1.8	4.3	0.9		
Urinary Calcium % total excretion ..	11—18%	5%†							9%	8%

* Some days before death, during intravenous calcium therapy.

† 50,000 units Vitamin D daily until 3 weeks before balance.

Table 4.

Aminoacids and other nitrogen compounds in plasma and urine of Lignac-Fanconi disease.

	Normal	Case 1 K.C.	Case 2 P.R.	Case 3 M.G.	Case 4 R.C.	Case 5 J.N.	Case 6 D.S.	Case 7 O.R.	Case 8 M.R.	Case 9 M.B.
Aminoacids in plasma by chromatography[1] ..	av. 52 (28—78)	74	120			75	>108		>74	93
Aminoacids in plasma by microbiology[2] ..	av. 16.4	24.8	26.4			19.4[3]			16.0[3]	
Aminoacids in urine by chromatography[1] ..	av. 19 (3—55)	125	145			92	95	66	106	111
Aminoacids in urine by microbiology[4] ..	up to 180	766	645						481	
$\frac{\text{Amino N} \times 100}{N_2}$ in urine	0.5—2	5—10	4—6	13		4	3.6		2—8	5
$\frac{NH_3.N \times 100}{N_2}$ during acidosis ..	>4	4—12	4—6	11—15		4—9	5.2—6.5		7—9	1
in urine during alkali therapy	2—4	4	2—5			3—5	6		3—6	0.5
Plasma albumin/globulin ratio ..	4—5/2—2.5	4.2/2.4	4.6/2.4	3.9/3.8		4.8/2.5	4.6/2.6		5.2/3.7	4.6/3.7
Plasma urea or N.P.N. mg./100 ml.	20—40	32⇄70	30⇄100	21—30	24→300*	25⇄122	16⇄75		26⇄78	80→285*

[1] Expressed as total sum of the colour intensity of the aminoacid spots from an arbitrary scale. A single representative chromatogram was chosen.

[2] Sum of 9 essential aminoacids, expressed in mg. per 100 ml. plasma.

[3] Sum of 6 essential aminoacids, expressed in mg. per 100 ml. plasma.

[4] Sum of 9 essential aminoacids, expressed as mg. in 24 hrs.

* Some days before death.

Except in the circumstances described above there is little evidence that the **parathyroid** plays an important part in this disease. Post mortem examinations by STURZENEGGER (1939), LOOSER (1944) and DRABLØS (1951) revealed parathyroids of normal size. Blood parathormone estimations by GUILD et al. (1937) and by GITTLEMAN and PINCUS (1940), using the method of Hamilton and Schwartz, showed normal values. Considerable and constant hypertrophy of the parathyroids would be reflected in excretion by the kidney of large amounts of calcium and phosphorus, while in fact the mineral disturbance is largely determined by intestinal malabsorption. Nevertheless, and especially in the chronic cases in which calcium deficiency has lasted for many years, compensatory hyperfunction of the parathyroids with some hypertrophy is to be expected and indeed has been demonstrated histologically by SCHIER and STERN (1926), BENOIT (1935), RUSSELL and BARRIE (1936), and RÖSSLE (1938) as well as in three of our cases. As in late rickets this may result in increased phosphate clearance (see Part 8). Table 3 shows the phosphorus and calcium levels in plasma and urine of nine of our patients.

3. Aminoacids, urea, ammonia and plasma protein

The **aminoaciduria** of Lignac-Fanconi disease may be strong enough to be detected by formol titration or by Folin's method. The results can be expressed conveniently as the ratio $\frac{\text{aminoacid N} \times 100.}{\text{total N}}$ The estimation is made normally on a single fasting urine specimen, as 24-hr. urine collections may be difficult. A coefficient of over 2 is pathological (PETERS and VAN SLYKE, 1946) and was found in all but one estimation made on eight of our patients. A coefficient of 10 or more was observed in Cases 1, 3 and 7 (Table 4).

A more sensitive and specific method of demonstrating the individual aminoacids is two-dimensional paper chromatography. Characteristic examples of urine and plasma chromatograms are given in Figs. 4 and 5. The greatly increased excretion in the urine of more than 10 aminoacids and the raised plasma level of various aminoacids is evident. A more detailed account of clinical chromatography and the results obtained in this series of cases is given in Part 7. Some observations which are of special clinical importance are recorded briefly here.

(a) The aminoaciduria in Lignac-Fanconi disease is generalised, with a pattern closely resembling that of the plasma. The high excretion of essential aminoacids is especially striking.

* Some days before death.

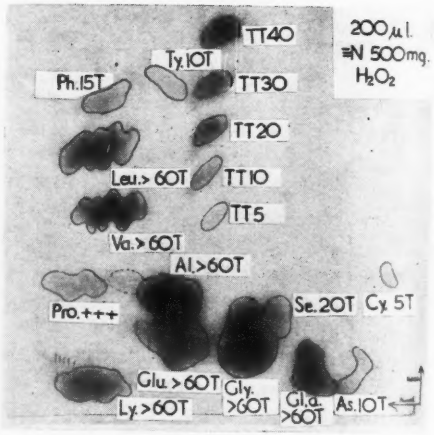


Fig. 4. Urine chromatogram of Case 13, showing strong general aminoaciduria. TT=test taurine. For other aminoacid abbreviations see Fig. 2, Part 1. Figures beside aminoacid names indicate colour intensity of spots as compared with taurine test spots (e.g. 10T=colour equal to 10 μg taurine).

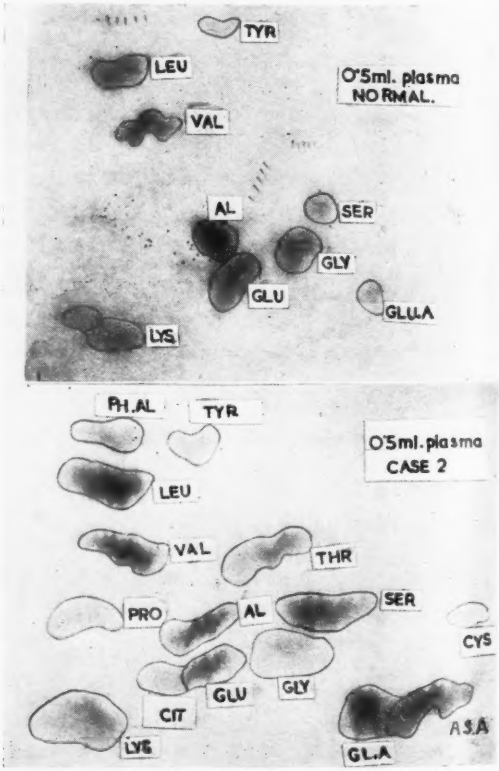


Fig. 5. Plasma chromatogram of Case 2 showing hyperaminoacidaemia, compared with a normal plasma chromatogram above.
(Volume used in both cases = 0.5 ml. deproteinised, desalted plasma.)

(b) In all our patients the pattern of the aminoaciduria shows little variability, but the degree of the aminoaciduria as a whole varies not only from case to case but from day to day in the same child and may even be completely absent for days on end.

(c) There is a rough relationship between the concentration of amino-acids in the urine and the severity of the disease. As a rule amino-aciduria is strong in the acute, infantile form of Lignac-Fanconi disease, such as Cases 1 and 2, but weak and easily overlooked in the chronic form of later childhood, such as Case 8.

(d) The concentration of aminoacids in the urine runs closely parallel to that of the plasma. This was shown in Case 2 in 21 plasma and urine chromatograms over a period of 8 months.

(e) Prolonged alkalinisation with sodium citrate led to cessation of aminoaciduria in all seven patients treated in this way.

(f) In certain plasma samples from our patients (e.g. Cases 2 and 6) the level of various aminoacids as shown by paper chromatography seemed to be raised. Paper chromatography, however, is no more than a semi-quantitative test. It has a marginal error of at least $\pm$ 20 per cent and is a method unsuitable for assessing slight variations from normal in the plasma levels of aminoacids.

We have, therefore, sought more accurate quantitative methods for the estimation of aminoacids in plasma. We are indebted to DR. SCHREIER of Heidelberg University and PROFESSOR KREBS of Sheffield University, who have carried out microbiological assays and glutamine estimations, using the glutaminase method. A detailed account of their results is given in Part 7. Microbiological assays by Dr. SCHREIER in Cases 1, 2, 5 and 8 showed an increase of various aminoacids in the plasma of between 50 and 100 per cent. In the urine the increase above normal of certain aminoacids was as much as twentyfold. The extent of the total increase in plasma and urine of nine essential aminoacids is shown in Table 4, which also gives an idea of the colour intensity of plasma and urine chromatograms in six of our patients. Professor KREBS found a similar increase in the plasma and urine content of glutamine and glutamic acid in Cases 1, 2, 8, 10 and 11. Estimations of the total aminoacid nitrogen in plasma by Folin's (1922) method were carried out repeatedly, but did not seem sufficiently sensitive or specific for the purpose.

Plasma urea values varied considerably in almost every patient (Table 4). In four of them levels of 100 mg. per cent or more were encountered, the highest level being 300 mg. per cent in Case 4 recorded

shortly before death. The generally accepted view that a rise in blood urea in Lignac-Fanconi disease is always due to glomerular insufficiency is not borne out by our investigations. Uraemia of that type was found only in Case 9, who was nearly ten years old, and possibly at an earlier stage in Case 4. In neither case, however, did post-mortem examination reveal the changes characteristic of true chronic uraemia (see Part 8). In all probability the raised urea in the other children was not the result of renal insufficiency. A urea value as high as 122 mg. per cent (Case 5) has been observed to fall to normal within a few days and remain normal for months. Some variations in blood urea level may be explained by the rapid alterations between dehydration and pre-oedema. But even without apparent dehydration high urea values are encountered, which at present we are unable to explain, though we are inclined to connect them with the disturbed aminoacid metabolism.

The extent of the **ammonia formation** in eight of our patients is seen in Table 4 and in Fig. 1, Part 7. Nearly all the ammonia values we have recorded are considerably lower than FANCONI's values in his Case 2, 1949, (Case 3 in this series) and in his earlier publications. Only in this case was the ratio $\frac{(NH_4)\ N \times 100}{\text{total N}}$ sometimes found to be over 10 (normal 2 to 4). In estimating the ammonia every effort has been made to avoid technical errors. Only freshly passed urine collected under paraffin was analysed ; the ammonia content was determined by the method of VAN SLYKE and CULLEN (1916), the aeration being carried out at room temperature to avoid formation of ammonia from glutamine and glutamic acid. The increased urinary ammonia in our patients seemed to depend mainly on the degree of acidosis and the functional efficiency of the kidneys, as may be seen from Table 4. High ammonia values in the urine of Cases 1, 3, 5 and 8 were associated with low CO_2 levels in the plasma. The urine of Case 9, however, showed a decreased ammonia coefficient despite acidosis, and this patient had the most advanced kidney destruction. During alkali therapy most of the raised ammonia coefficients fell to normal levels. Fig. 1 in Part 7 shows the ammonia production in Cases 1 and 8 on consecutive days during a normal period, under acid feeding and under alkali feeding. There is an obvious correlation between the degree of acidosis and the ammonia production, but the ammonia production during the period when acids were administered seems to be less efficient than in a normal child, especially in the chronic Case 8 with advanced kidney dysfunction.

To summarise : the aminoacid, urea and ammonia investigations show that in Lignac-Fanconi disease the aminoaciduria is associated with an aminoacid plasma level raised by 50 to 100 per cent and that this aminoacid increase in blood and urine is accompanied by normal or increased formation of urea and, as a rule, of ammonia.

There is, therefore, no reason to doubt the body's capacity for adequate deamination and one is rather led to assume that the aminoacid disorder lies in the direction of protein synthesis. The importance of more detailed investigation of the plasma protein in this disease becomes apparent. Unfortunately the scope of such investigation is at present limited, but new methods of research are now available. These include tracer work with labelled aminoacids and the analysis of protein hydrolysates by means of microbiological and special chromatographic methods (starch and resin columns by MOORE and STEIN, 1948, 1949).

The **total plasma protein** content in seven of our patients was normal and the fibrinogen levels normal or slightly raised (up to 0.67 per cent). The albumin-globulin ratio, when estimated by the ordinary chemical methods, was within normal limits in Cases 1, 2, 5 and 6, whereas Cases 3, 8, and 9 showed an increase in the globulin and Cases 10 and 11 decrease in the albumin fraction. The more delicate electrophoretic differentiation of the plasma protein in Case 3 by Wuhrmann and Wunderly (FANCONI and BICKEL 1949), and in Cases 10 and 11 by Martin (see PHILPOTT, HARVEY and FINCH, Part 6) showed an increase of the α-globulin and a somewhat smaller increase of the β-globulin. Drs. HARDWICKE and STANWORTH of the Department of Experimental Pathology, Birmingham University, examined serum from Cases 1, 2 and 8 without finding gross differences between these and the results obtained on two normal children. This may be due to the fact that these patients were already improving under treatment. HARDWICKE'S and STANWORTH'S findings are summarised in an addendum to this paper. The changes in the globulin of Cases 3, 10 and 11 are of special clinical interest as a possible explanation of the high B.S.R. readings of many of these children and their reduced resistance to infections, such as measles, to which they so often succumb. It is possible that the globulin changes originate in a disturbance of the reticulo-endothelial system.

A first rough insight into the aminoacid structure of the plasma protein of four of our patients (Cases 1, 2, 5 and 8) was achieved by examining plasma hydrolysates by means of paper chromatography. The aminoacid pattern was found to be exactly the same as that of a normal plasma hydrolysate (Fig. 6). This finding suggests that the metabolic disorder is not restricted to one or two aminoacids only but is generalised.

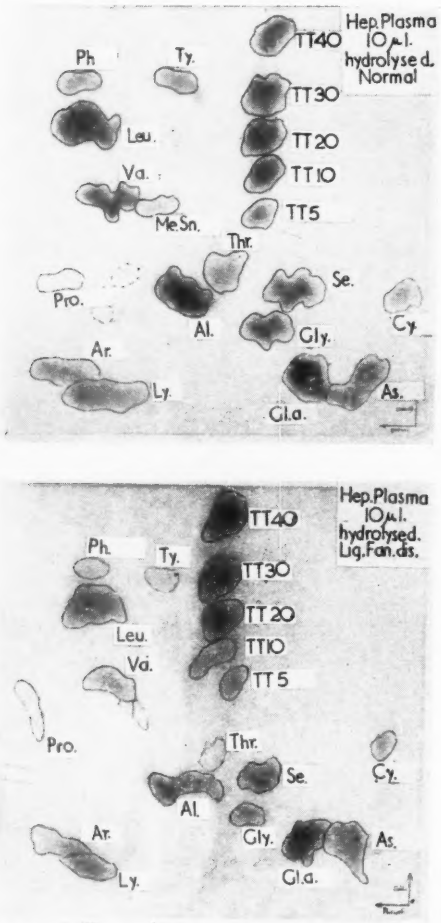


Fig. 6. Chromatogram of hydrolysed plasma protein of Case 2, compared with normal plasma hydrolysate (above).

4. **Acid-base and electrolyte findings**

Acidosis indicated by a low CO_2-combining power in the plasma was present in all our patients. Its intensity appeared to correspond to the severity of the disease. In the acute, severely ill Cases 1 and 2 the lowest CO_2 values obtained were 8.1 and 7.2 mEq/l per cent, wnile in the chronic and mild Cases 7 and 8 they did not fall below

Table 5.

Some plasma and urine electrolytes in 7 cases of Lignac-Fanconi disease.

	Normal	Case 1 K.C.	Case 2 P.R.	Case 3 M.G.	Case 5 J.N.	Case 7 O.R.	Case 8 M.R.	Case 9 M.B.
PLASMA								
Sodium mEq/l	137—145	128—136	163	130—147	141		132—142	145—117[2]
Potassium mEq/l[1]	3.8—5.0	2.3—4.2	2.5—4.7		2.7—2.8		1.8—3.1	
Chloride mEq/l	98—106	97—122	89—115	97—107	102—107	102—107	90—131	102—65[2]
CO_2–combining power mEq/l	22—28	7.8—20.3	7.2—23.4	15.3—16.7	12.6—18.0	21.6—24.0	21.6—23.9	9.9—22.5
URINE								
pH during acidosis	<6	6.0—8.2	6.8—7.6	5—7.8	6.2—7.0	7.3	5.6—6.8	6.8—7.0
Bicarbonate during acidosis mEq/kg/24 hrs.	Nil	1.1—4.4	1.4—3.9		3.5		0.1—1.0	
Organic acids mEq/kg/24 hrs.[3]	1	2.1—4.8	4.2—7.0		9.5		1.7—2.7	1.4

For calcium and phosphorus see Table 3, proteins and aminoacids see Table 4.

[1] All potassium estimations were carried out by Drs. Barclay and Ibrahim, Dept. of Physiology, The University of Birmingham, by flame photometer.

[2] Values shortly before death, influenced by suprarenal haemorrhages.

[3] Values obtained by the "indirect method" (see text Part 7, page 158).

21.6 mEq./l (Table 5). In each individual patient the degree of the acidosis varied considerably apart from treatment, and normal or only slightly lowered CO_2 values were found occasionally in almost all. Isolated estimations of CO_2-combining power do not, therefore, form a proper basis for conclusion, any more than do isolated urea and phosphorus estimations in the plasma or isolated tests for aminoacids or sugars in the urine.

The chronic and often serious acidosis of Lignac-Fanconi disease is certainly of importance in its effects on the course of the disease and its treatment. This is borne out by the following clinical observations :
(a) Serious metabolic crises in Cases 1, 2, 3, 5 and 9 were accompanied by a rapid fall in the CO_2-combining power. They ceased when thorough alkalinisation had been achieved and the CO_2-combining power had become normal.
(b) After some weeks of alkalinisation with correction of acidosis the aminoaciduria disappeared and the glycosuria decreased or disappeared (Cases 1, 2, 5, 6, 8, 13).
(c) Two patients with the acute form of Lignac-Fanconi disease (Cases 1 and 2), while fully alkalinised, survived measles and other infections without causing any real anxiety. Infections such as these are known to be dangerous and often fatal in untreated cases of the disorder.

In order to learn more about the nature of the acidosis we studied in detail the electrolyte structure of blood and urine in two of our patients (Cases 1 and 8) under normal conditions and also after feeding with small amounts of acid. These studies were suggested by the publication of LINDER, BULL and GRAYCE (1949), who were the first to investigate in detail the acid-base metabolism in this disease.

Our results corroborate and amplify their findings and will be described in detail in Part 7. Plasma and urine ionograms of these children are given in Figs. 7 and 8. Here follows a short account of some findings which are of clinical importance :

(a) A well-marked **hypo-electrolytaemia** of 272 mEq/l at its lowest level (normal about 300 mEq/l) was found in the acidosed Case 1 and was shown to be due to excessive loss of electrolytes in the urine, namely 32 to 39 mEq/Kg/24 hrs. Case 8 was not acidosed at the time of the test and did not show hypo-electrolytaemia, though there was also some excess of electrolytes in the urine (20 to 25 mEq/Kg/24 hrs.). Normal 4 year old children, despite a higher electrolyte intake due to better appetite, excrete about 15 to 18 mEq/Kg/24 hrs. (Macy 1942).

(b) **Loss of fixed bases and chloride in the urine.** As the excretion of these electrolytes is largely dependent on the intake, a pathological loss of sodium, potassium and chlorides is only present if the intake is not increased in the patient as compared with a healthy control. In both Cases 1 and 8 the intake, due to the

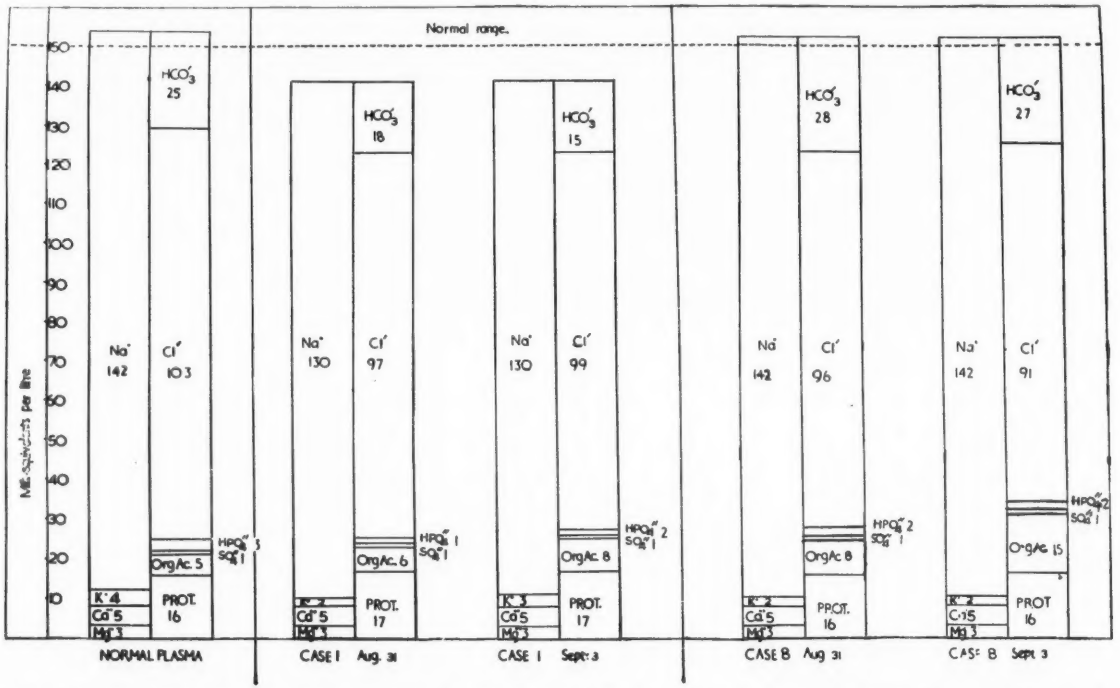


Fig. 7. Plasma ionograms of two cases of Lignac-Fanconi disease (Cases 1 and 8) and of normal plasma. Expressed as mEq./l.

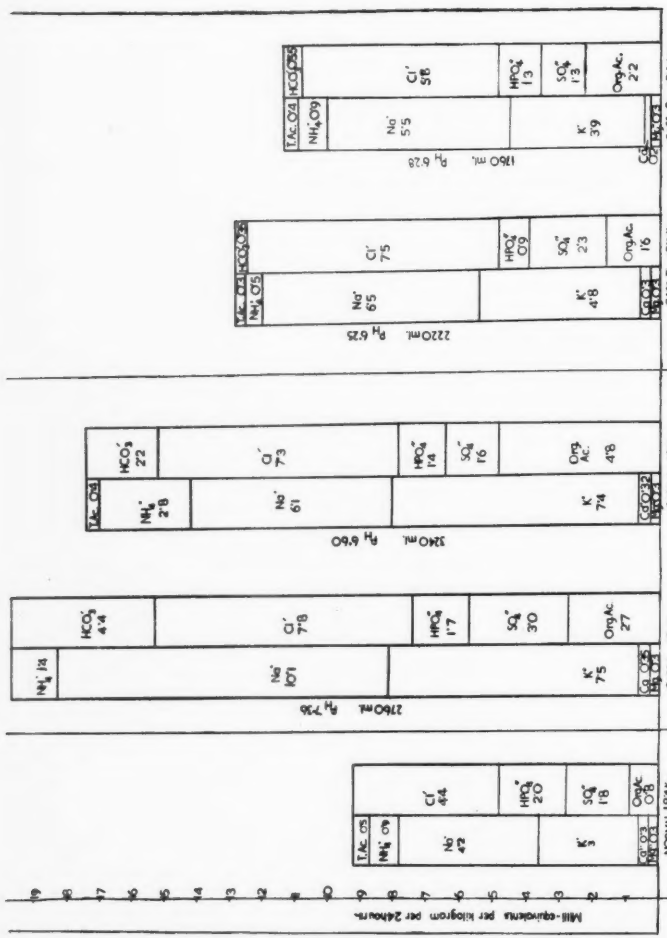


Fig. 8. Urine ionograms of two cases of Lignac-Fanconi disease (Cases 1 and 8) and of average values as found in normal children (Macy 1942). Expressed as mEq./kg./24 hrs.

anorexia of the patients, was considerably below the normal average (for details see Part 7). The increased loss of fixed bases was thus pathological and was the main factor in the hypo-electrolytaemia on the base side. The loss of fixed bases in Case 1 reached 18 mEq/Kg/24 hrs. (normal 6 to 8 mEq/Kg/24 hrs.). Sodium and potassium were affected similarly, and this was reflected in their lowered concentration in plasma. The hypopotassaemia was especially striking, with values as low as 2.3 mEq/l in Case 1 and 1.8 mEq/l in Case 8 (normal 3.8 to 5.0 mEq/l).* The sodium plasma values were low in Case 1 (lowest value 124 mEq/1, normal 137 to

* These potassium estimations were made by DRS. BARCLAY and IBRAHIM by flame photometer

145 mEq/l) but normal in Case 8. The loss of fixed bases was accompanied by an increased loss of chloride in the urine (up to 7.8 mEq/Kg/24 hrs. in Case 1, 7.5 mEq in Case 8, normal 4 mEq) and low normal or slightly decreased plasma levels. In evaluating these figures allowance must be made for the state of hydration. During the phases of dehydration which are frequently encountered in this disease, normal or even raised blood levels for potassium, sodium and chlorides may be found and are misleading. The wide range of results obtained in the estimations of these ions reported in Table 5 and in the literature of the disease reflects in some measure this effect of dehydration.

(c) **Loss of bicarbonate in the urine.** Cases 1 and 2, and also Cases 10 and 11 (see PHILPOTT, HARVEY and FINCH, Part 6) excreted large quantities of bicarbonate in the urine, despite acidosis with plasma CO_2 values of 14.5 mEq/l and less. This behaviour is pathological (see Part 7) and explains the abnormally high pH (up to 8.14) in the urine of such patients. The abnormal loss of bicarbonate was not a constant feature of all our patients (see Table 5). Five excreted an acid urine with a pH of less than 6 when acidosed and this excludes any appreciable loss of bicarbonate. Furthermore, the bicarbonate loss in Case 1 stopped completely during a period when he was given 2 grams calcium chloride daily ; at the same time the urine pH dropped from 8.14 to 5.38, while the plasma CO_2 fell to the alarmingly low level of 7.3 mEq/l. A normal control child was not in any way affected by the small dose of calcium chloride.

(d) **Ammonia formation and titratable acidity** (buffer substances). These two important defence mechanisms against acidosis were found to be defective in Case 1 and even more so in Case 8. The response to acid feeding in the production of ammonia and buffer substances was either inadequate or completely absent (Part 7). Instead there was a relatively high excretion of fixed bases which buffered the high excretion of acid radicles such as organic acids and bicarbonate. The lowered titratable acidity in Case 1 may be explained in part by the decrease in the excretion of phosphate during the period of acid feeding (Part 7). The ammonia findings in other patients have been mentioned above (page 44).

(e) **Organic acids in urine and plasma.** These were measured by an indirect method (see Part 7). In five of our patients (Table 5) every urine specimen examined showed a well-marked increase of between 1.4 and 9.5 mEq/Kg/24 hrs. (normally less than 1 mEq/Kg/24 hrs.). The levels of organic acids in plasma are difficult to determine for technical reasons (see Part 7) and figures for normal subjects are not available. It is highly probable that values of 12.3 mEq/l obtained in Case 1 and 15.2 mEq/l in Case 8 are high. On the days that these estimations were made there was no ketonuria in these patients.

In conclusion, the acidosis of Lignac-Fanconi disease is due to dysfunction of various mechanisms which under normal conditions ensure the iso-ionia of the plasma. There is abnormal bicarbonate loss, relatively poor ammonia formation in the kidney, and decreased buffer capacity of the urine, the result of which is an increased excretion of fixed base. However, it is unlikely that these factors alone are responsible for the acidosis. Some patients described in this series and earlier who were markedly acidosed showed no bicarbonate loss and a good

formation of ammonia (ammonia coefficient of 15 in Case 3). On the other hand an increased excretion of organic acids in the urine was demonstrated in all patients, and we have adduced some evidence that the blood level also was raised. It is unlikely that these organic acids are aminoacids, as even a hundred per cent increase of the aminoacid blood level would account for less than 1 mEq/l organic acids. Nor are they keto-acids since no ketone bodies were present in the urine at the time the test was made. Our hypothesis for their origin will be given in Parts 7 and 8. It is possible that they are of importance in damaging the kidney and causing its progressive destruction.

The hypopotassaemia is of special clinical interest and has an important role in Lignac-Fanconi disease. In addition to Cases 1 and 8, whose disturbed potassium metabolism has been described above, hypopotassaemia was observed in two other patients (Cases 2 and 5) in this series, in a patient of Professor WATKINS and Dr. R. J. K. BROWN in Cardiff (personal communication), in a patient of Drs. PAYNE and BLACK in The Hospital for Sick Children, Gt. Ormond Street, London (personal communication) and by DRABLØS in his recent publication (1951). Hypopotassaemia is manifested in symptoms such as vomiting, muscular weakness and hypotonia, constipation, cyanosis, peripheral vascular collapse and toxic crises, which are relieved considerably by the administration of potassium salts (see case records 1, 2 and 8 and p. 75). Hypopotassaemia also causes electrocardiographic changes, which are of diagnostic importance inasmuch as they confirm the results of chemical analysis. Unless a flame photometer is used, evidence of potassium deficiency is demonstrated more rapidly by electrocardiography than by chemical analysis. We are grateful to Dr. C. G. PARSONS for the following resumé of his studies on three of our patients (Cases 1, 2 and 8).

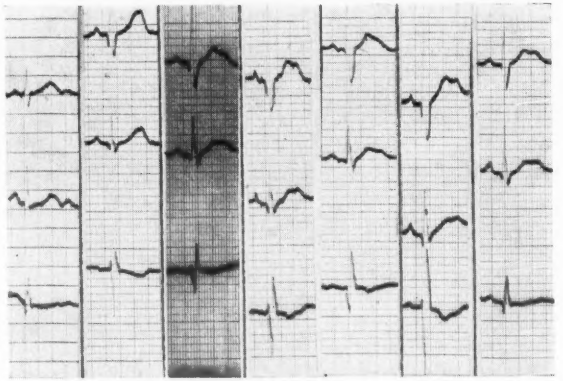
Electrocardiographic Changes

Tracings were made with an Elmquist Triplex electrocardiograph in which time-markings are drawn 0.10 sec. apart. The duration of the R-R interval has been taken as the average of all beats recorded in the full length of each tracing. The Q-T intervals have been measured in the lead showing the largest and most clearly defined T waves, and the longest interval has been chosen. Q-T measurements have then been corrected for cycle length (BAZETT's formula 1918-1920) using a nomogram devised for this purpose by KISSEN, SCHWARZSCHILD and BAKST (1948). In addition to Q-T_c the Q-T ratio has been calculated from the measured R-R and Q-T intervals by means of a nomogram (GOLDBERGER, 1949). Figures for normal children published by ASHMAN and HULL (1941) give as the upper limits 0.422 sec. for Q-T_c and 1.08 for the Q-T ratio.

The results obtained in three children suffering from Lignac-Fanconi disease are summarised in Table 6.

Table 6a.

REPORT ON ELECTROCARDIOGRAMS of Case 1. Age 3 Y.



	23.2.51	29.8.51	4.9.51	5.9.51	8.9.51	11.9.51	15.9.51
K.mEq./l	—	5.4	—	2.2	2.1	3.6	2.8
Rate/min.	159	100	111	120	107	125	108
R–R (sec.)	0.38	0.60	0.54	0.49	0.56	0.47	0.55
Q–T (sec.)	0.26	0.30	0.32	?0.30	0.32	0.33	0.33
Q–T_c (sec.)	0.415	0.388	0.435	?0.430	0.425	0.475	0.445
Q–T Ratio	1.05	0.96	1.08	?1.08	1.05	1.20	1.10

Upper normal limit 0.422

Upper normal limit 1.08

Remarks

23.2.51 T_1 and T_2 domed ; T_3 biphasic.

29.8.51 Dehydrated. T_1 and T_2 domed ; T_3 inverted. Left axis deviation.

4.9.51 T_1 and T_2 with plateau tops. T_3 flat. RS-T_1 and 2 rising abruptly.

5.9.51 T_1 and T_2 with plateau tops ; T_3 inverted. S-T slightly depressed. Left axis deviation.

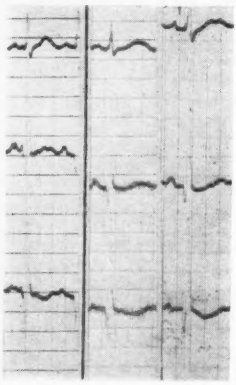
8.9.51 T_1 and T_2 with plateau tops ; T_3 inverted. RS–T abnormal shape in all leads.

11.9.51 T and RS–T changes as before but less severe. R–T_3 depressed.

15.9.51 T waves still abnormal but T_3 now upright.

Table 6b.

REPORT ON ELECTROCARDIOGRAMS of Case 2. Age 3 Y.



	23.2.51	1.5.51	4.5.51
K.mEq./l	—	—	—
Rate/min.	154	104	130
R–R (sec.)	0.39	0.57	0.46
Q–T (sec.)	0.27	0.34	0.33
Q–Tc (sec.)	0.430	0.385	0.480
Q–T Ratio	1.08	1.13	1.22

REMARKS

23.2.51 Slight plateau top T_1 ; T_3 inverted.

1.5.51 T waves flatter. T_3 and V_1—V_3 inverted.

4.5.51 T_1 flat topped ; T_3 inverted ; S–T depressed in I., II. and III. ; T lower voltage ; T is inverted in V_1—V_4 and flat in V_5—V_6.

Although not constantly present, three abnormalities occur at various times in the electro-cardiograms of each child :

1. prolongation of electrical systole ; 2. changes in the T waves ; 3. displacement of the RS-T segments. Careful measurements revealed no significant changes in the shape, size or duration of P, Q, R, S or U waves, and no noticeable variation in the duration of the P-R intervals.

Prolongation of the Q-T interval occurs in rheumatic carditis, hypopotassaemia and hypocalcaemia. It is seen also in renal failure. T waves, which are often large in healthy children, may be flattened or inverted in myocarditis and in hypopotassaemia. Similar changes in diabetic acidosis and chronic nephritis are probably due to hypopotassaemia, since hypocalcaemia has no effect on the size or shape of the T waves.

The RS-T segment is depressed in hypopotassaemia, and elevated in such conditions as pericarditis and coronary artery obstruction so long as inflammation is active or necrosis occurring. The "current of injury" which leads to this RS-T elevation can be reproduced by applying potassium chloride to the surface of a muscle cell.

From the above considerations it follows that the low serum potassium characteristic of Lignac-Fanconi disease could account for the electrocardiographic abnormalities. It is unwise to draw conclusions from so small a number of observations, but in the children studied depression of the RS-T segment is the least constant, and deformity of the T waves the most constant abnormality. Alteration in the shape of the T wave seems to be a more delicate indicator of

Table 6c.

REPORT ON ELECTROCARDIOGRAMS of Case 8. Age 7 Y.

	22.2.51	29.8.51	4.9.51	5.9.51	8.9.51	11.9.51	15.9.51
K.mEq./l	—	2.0	—	1.7	2.0	3.3	3.6
Rate/min.	102	98	91	102	91	112	92
R–R (sec.)	0.58	0.61	0.65	0.58	0.66	0.53	0.63
Q–T (sec.)	0.34	0.37	0.38	0.36	?	0.32	0.38
Q–Tc (sec.)	0.446	0.470	0.470	0.470	?	0.438	0.478
Q–T Ratio	1.10	1.18	1.17	1.18	?	1.10	1.19

	Remarks
22.2.51	T with plateau summit in standard limb leads I and II ; inverted in III ; plateau summit in V_1.
29.8.51	T waves flat in standard limb leads ; plateau summits.
4.9.51	Little change in T waves. Somatic tremor.
5.9.51	T with plateau top in I and II ; inverted in III. $S–T_2$ depressed.
8.9.51	As on 5.9.51 ; marked tremor.
11.9.51	T_1 and T_2 flat topped. T_3 biphasic with slightly abnormal RS–T segment.
15.9.51	T waves taller but still with changes of 11.9.51. $RS–T_2$ and $_3$ depressed.

hypopotassaemia than a reduction in the voltage of the wave. As serum potassium falls, the summit of the wave at first loses its point and becomes domed. Later the top of the wave flattens into a plateau or there are two peaks separated by a flat segment. Reduction in voltage follows, but flattening of the top of the wave remains. As the level of potassium is restored the order of these changes is reversed.

Changes in the shape of the RS-T segment are probably characteristic also but they are less readily recognised than the T wave deformities and in consequence are less important. In potassium deficiency the ascent to the T wave seems to be more abrupt, following closely after the QRS but separated from it by a distinct iso-electric phase. The RS-T segment is therefore convex downwards when the T wave is upright, and convex upwards when the T wave is inverted. (See Lead III of Case 1 on September 8, 11 and 15, Table 6a). T wave and RS-T changes can be appreciated in any lead, but they are more readily recognised in the standard than in the unipolar limb leads, and only very occasionally could they be seen most clearly in the chest leads. For these reasons only the standard limb leads are illustrated in the table.

Prolongation of the Q-T interval was a constant observation whilst Case 8 (Table 6c) had hypopotassaemia, but was inconstant in Case 1. In both children the Q-T interval showed an unexpected increase in duration as the serum potassium rose toward normal and, although additional electrocardiograms might have shown an eventual return of Q-T to normal, doubt is cast on the sensitivity of the Q-T interval as an indicator of potassium levels in the serum. Hypopotassaemia is often associated with a prolonged Q-T interval, but when the serum potassium level is restored the duration of electrical systole returns to normal slowly, probably being delayed longer than an improvement in the shape of T waves.

5. Glycosuria and sugar tolerance tests in Lignac-Fanconi disease

The original publications of LIGNAC (1924) and FANCONI (1931) called attention to the disturbance of the sugar metabolism in this disease. Most workers have identified the reducing substance present in the urine as glucose and have shown that the glycosuria is renal in origin. This is true of all our cases. Others, however, have reported the finding of a variety of sugars—pentose, lactose, fructose and glucuronic acid, while the results of some blood sugar tolerance tests have been pathological and quite atypical of renal glycosuria. Most alarming was the collapse, sometimes profound, which developed in

some patients during a glucose tolerance test. DEBRÉ's patient (1934) actually died during one of these attacks.

Glycosuria. This was present in 13 of our 14 patients, but was rarely more than slight (0.1 to 1.2 grams per cent, see Table 7), and easily overlooked in the highly diluted urine. In the chronic form of the disease especially, routine tests for sugar were negative for days or weeks on end, while in the acute form glycosuria, like aminoaciduria, was more intense. Under long-continued alkali therapy glycosuria, like aminoaciduria, is reduced and may finally disappear.

For the detection of only mild glycosuria Benedict's test is unsatisfactory, and we recommend the much more sensitive and specific method of sugar chromatography (PARTRIDGE and WESTALL 1948, HORROCKS and MANNING 1949), which may reveal pathological amounts of glucose even in urine specimens which give a negative Benedict's test (see Fig. 9). To establish the pathological significance of these traces of sugar, the urine of one hundred healthy children was examined by sugar chromatography but no sugars were detected (unpublished work).

Blood sugar tolerance test. The results of oral glucose tolerance tests in six of our patients are shown in Table 8. The amounts given as a single dose varied from 6.5 to 50 gms. In the fractional test in Case 3 it consisted of three doses of 20 gms. each, given at hourly intervals. Normally hyperglycaemia of 160 to 200 mg. per cent

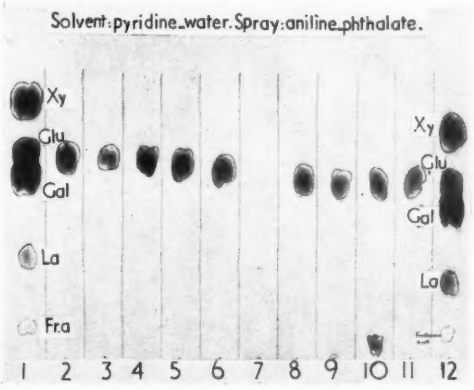


Fig. 9. Sugar chromatogram showing glucose excretion in the urine of 9 patients with Lignac-Fanconi disease. For purposes of comparison pure lactose, galactose, glucose, fructose and xylose were run in the two outer columns and a normal urine in column 7. Columns 2 Case 9; 3 Case 14; 4 Case 13; 5 Case 12; 6 Case 1; 8 Case 2; 9 Case 7; 10 Case 10; 11 Case 11.

Table 7.

Blood sugar, urine sugar and acetone in 9 cases of Lignac-Fanconi disease.

	Normal	Case 1 K.C.	Case 2 P.R.	Case 3 M.G.	Case 4 R.C.	Case 5 J.N.	Case 6 D.S.	Case 7 O.R.	Case 8 M.R.	Case 9 M.B.
Fasting blood sugar mg%	75—110	80—105	74—200†	40—90	—	85	84—138	—	88	97—417*
Sugar g.%	0	0—1.2	0—0.36	0—0.9	0	0—0.23	±	0—0.12	0—0.6	0—0.25
Sugar qualitative		glucose	glucose	glucose		glucose	glucose	glucose	glucose	glucose
Acetone	—	±	±	—	—	±	±	±	—	±

† During metabolic crisis.

* Shortly before death.

Table 8.

Oral sugar tolerance tests in 6 cases of Lignac-Fanconi disease.

Case No.	Weight kg.	g. Sugar ingested	Fasting	½ hr.	1 hr.	1½ hrs.	2 hrs.	2½ hrs.
1	7.5	15	80	127	135	110	106	99
2	8.6	15 40 50	100 80 102	220 220 —	114 241 242	113 220 —	102 128 144	91 — 74
3	6.5	3×20	85	150	160	170	180	140
6	10.5	6.5	138	164	170	186	120	138
10*	15.7	20	101	110	128	—	138	106
11*	16.4	20	94	110	154	185	192	127

* Cases of Philpott, Harvey and Finch, Part 6.

maximum should not be exceeded, even when high doses are given (PETERS and van SLYKE 1946). In Case 2 this level was passed in each of three tests, while Cases 3, 6 and 11 reached the upper limit of normal. Blood sugar values should normally return to the fasting level in 2 hours, though if large doses of glucose are given this time may be exceeded by an hour or two even in health. The high dosage we used may account for the slow return of blood sugar levels to normal noted in Case 2, second and third test, and in Case 3, while Cases 10 and 11 showed pathologically prolonged curves. In Case 3, moreover, the absence of the usual counter regulations to fractional doses is remarkable.

The high and prolonged oral blood sugar curves noted in several of our cases correspond to the observations of DE TONI 1933, DEBRÉ 1934, FANCONI 1936, 1946, DANIS and ROSSEN 1941 and MCCUNE, MASON and CLARKE 1943. In marasmic children with Lignac-Fanconi disease this abnormal response to sugar ingestion is possibly due to starvation.

There is experimental evidence that after prolonged fasting the ingestion of sugar causes an abnormally high and prolonged tolerance curve (SHOPE 1927, SWEENEY 1927, GOLDBLATT and ELLIS 1932). This results from impaired combustion of sugar in the tissues during starvation and is more pronounced in infancy than in later life (LIVINGSTONE and BRIDGE 1942) due to poor economy in the expenditure of carbohydrate stores in infancy. Furthermore, carbohydrate starvation as a factor in Lignac-Fanconi disease is evident in the hypoglycaemic fasting blood sugar levels which are occasionally found in this disease, and also in ketonuria, which was observed from time to time in most of our patients.

Collapse during glucose tolerance tests. These shocks are characterised by rapid and severe peripheral vascular failure and vomiting. They are dangerous and may be fatal and have been observed only by DEBRÉ (1934), FANCONI (1936, 1946) and FANCONI and BICKEL (1949) in Case 3 of the present series, but in none of our other patients. This may be attributed in part to the large test doses of sugar used by these workers. The recorded cases of shock with hyperglycaemia in association with glucose tolerance tests are as follows :

Author	Case	Glucose dose in grams.	Highest blood sugar level mg.%
DEBRÉ (1934)		50	385
FANCONI (1936)	Case 2		
	1st test	3×15	270
	2nd „	3×15	250
	Case 3		
	1st test	3×20	215
	2nd „	3×20	170
	3rd „	3×20	230
FANCONI (1946)	Case 2	3×20	240
FANCONI and BICKEL (1949) (Case 3 of present series)	Case 2	3×20	180

The injection of adrenalin (0.2 to 0.3 ml. of the 0.1% solution subcutaneously), customary in Fanconi's hospital after the third dose of glucose, revealed a marked sensitivity to adrenalin in patients with Lignac-Fanconi disease and resulted in hyperglycaemic values of as high as 380 mg. per cent.

The cause of the collapse, when it occurs, is, we believe, neither hyperglycaemia nor increased acidosis, but a sudden fall in the plasma potassium level. If, as frequently happens in this disease, the potassium level is already low, the further fall occasioned by the administration of glucose may bring about hypopotassaemia sufficient to cause collapse. The influence of oral glucose on blood sugar and potassium has been investigated repeatedly in one of our patients (Part 7, Fig. 2). and a considerable decrease of the plasma potassium level has been observed consistently. The explanation of this behaviour lies in the close interrelationship that exists in the cellular metabolism of potassium and sugar (see Part 7, p. 167).

The mechanism of glycosuria in Lignac-Fanconi disease

In all our cases the glycosuria was "renal," that is, it was usually associated with normal levels of blood sugar, which in seven of our patients was shown by chromatography to be glucose. The mechanism of this "renal glycosuria" is not yet clearly understood.

In view of the normal blood sugar level two possible explanations are suggested. FANCONI (1936) thought that the faulty reabsorption of glucose was the result of inadequate phosphorylation which in turn was due to phosphatase reduction in the tubules. This reduction has in fact been demonstrated by STOWERS and DENT (1948) in their adult patient with "Fanconi's syndrome" using Gomori's staining technique, and by BAAR in one of our patients with cystine storage disease (see Part 8). Besides the kidneys, BAAR has found reduction of the phosphatase in liver, bone marrow and spleen of the same child. He has also seen decreased phosphatase staining in the kidney of a patient suffering from ordinary glomerulo-tubular nephritis. We conclude that the phosphatase depletion seen in kidney tubules in Lignac-Fanconi disease is not limited to the kidney alone nor is it specific for this disease. It may be the result either of tissue damage in a generalised disorder or of a wide-spread phosphatase deficiency. Renal glycosuria may be explained by the phosphatase depletion in the tubules. The other possible explanation of the renal glycosuria is that the glucose is formed in the kidneys themselves from the excess of aminoacids present in Lignac-Fanconi disease. Evidence has accumulated that the kidneys are able to form glucose from aminoacids. RUSSELL and WILHELMI (1941) were able to show that kidney slices transform alanine and glutamic acid into carbohydrates.

6. The function of liver and kidneys

In Lignac-Fanconi disease the function of the liver and kidneys, like their histology, shows wide variability from normal to severely abnormal. In the advanced stages of the disease the severe tubular and glomerular lesions and the general atrophy of the kidneys necessarily disturb their function. In the early stages of the disease histological changes are absent (HOTTINGER 1947, DRABLØS 1951) or no more than slight (STURZENEGGER 1939), and are in keeping with the absence of, or slight, functional disturbance. Similarly in the liver parenchyma the appearances vary from normal structure to advanced fatty infiltration, focal necrosis and other evidence of degeneration "not dissimilar to that presented by the kidneys" (RÖSSLE 1938). It is reasonable to assume that the degree of functional disturbance of the liver varies accordingly.

(a) **Renal function** (Table 9). Case 7 in our series was diagnosed unusually early, showed the least constitutional upset and was found to have no more than minimal disturbances of kidney function. In the urine traces of albumen and glucose were found only occasionally. The centrifuged deposit contained a few pus cells. Specific gravity ranged from 1004 to 1024. Urine volume and ammonia production were normal. On the other hand, the chronic Cases 8, 9, 10 and 11 showed much more severe kidney dysfunction. Albuminuria was heavier and more continuous. In the centrifuged deposit hyaline and granular casts were found, as well as pus cells and erythrocytes, though

in no great numbers. Ammonia production was inadequate, particularly in the chronic Cases 8 and 9. Poor bicarbonate reabsorption was observed in the chronic (Cases 10, 11) as well as in the acute form (Cases 1, 2), and caused the high pH in the urine of these patients (see p. 51).

The specific gravity of the urine varied between 1005 and 1010 in Case 8, 1005 and 1011 in Case 9 and 1013 to 1014 in Case 11. Decrease in the fluid intake reduced the urine volume only slightly (Cases 3, 8). The polyuria, which was often considerable, was not influenced by pitressin (Cases 4, 6, 14), nor did it seem to be closely related to the degree of kidney destruction. The chronic cases with advanced kidney insufficiency excreted normal daily volumes of urine (Case 9 1100 ml., Case 11 1100 ml.) without developing oedema, while the younger acutely ill children (Cases 1 and 2) often passed more than 3 litres daily.

In the late stages of the chronic form there is increasing nitrogen retention and this must be differentiated from the raised blood urea of the early stages of Lignac-Fanconi disease, which is temporary and probably extrarenal in origin. Even in the chronic cases blood pressure generally remains normal. The highest reading, 110/70, was recorded in the oldest member of this series. In none of our cases was there any pathological change in the optic fundi. Plasma cholesterol levels of 482 and 310 mg. per cent were found in Cases 9 and 11 respectively and were considered to be a good indication of the degree of renal insufficiency present in those patients.

Few clearance tests were attempted because of the technical difficulties in young children. In Case 8 the urea clearance was 43 per cent of normal, in Case 9 38 per cent, in Case 11 33 and 45 per cent, while in the less advanced Case 10 it was normal (96 and 76 per cent, all results corrected for body weight). The phenolsulphonephthalein excretion was tested in Case 14 and gave a figure of 30 per cent in 2 hrs. compared with a normal of 60 per cent or more. LINDER (1949) found reduced phthalein and normal urea clearance simultaneously in his 13 year-old chronic patient and concluded that "this emphasizes the tubular nature of the lesion, for urea is not excreted by the tubules," whereas for phenolsulphonephthalein "SMITH (1937) calculated that 6 per cent of the dye excreted is filtered by the glomerulus and 94 per cent is excreted by the tubules." Unfortunately, cystine storage was not demonstrated in Linder's case, so that the diagnosis of Lignac-Fanconi disease was not firmly established. The need for many more observations of clearance

Table 9.

Various urinary findings, blood pressure, plasma urea and cholesterol in Lignac-Fanconi disease.

	Normal	Case 1 K.C.	Case 2 P.R.	Case 3 M.G.	Case 4 R.C.	Case 5 J.N.	Case 6 D.S.	Case 7 O.R.	Case 8 M.R.	Case 9 M.B.
Urine vol. in 24 hrs. (ml.)	av. 950	1200—3200	1000—3000	500—600		1200—2000	1430 and more	intake av. 900	1300—2200	1100
Specific gravity ..	1001—1030	1010—1012	1003—1015	1003—1011		1001—1004	1002—1010	1004—1020	1005—1010	1005—1011
Albumen g.% ..	0	0—0.07	0—0.25	0—0.29	+	0—0.12	0—0.05	(±)	0—0.17	0—0.34
Centrifuged deposits pus cells ..	—	(+)	(+)	(+)	(+)	(+)	—	(+)	(+)	(+)
R.B.C. ..	—	—	—	—	(+)	—	—	—	(+)	(+)
casts ..	—	(+)	—	—	—	—	—	—	—	(+)
Blood pressure mm.Hg.	100/60	105/65	85/65	100/55	60/25	80/50	90/55		90/65	110/75
Plasma urea or N.P.N. mg.%	20—40	32⇄70	30⇄100	21—30	24→300*	25⇄122	16⇄75		26⇄78	80→285*
Plasma cholesterol mg.%	90—180	107—173	178—248	350—430	182	200			135—233	267—482

*Values shortly before death.

tests is obvious, especially in the early stages of the disease. Here DRABLØS' (1951) contribution is most valuable, for in his 18 months-old patient without a histological kidney lesion he reported urea clearances of 90 and 77 per cent and inulin clearances of 32 and 51 per cent.

The degree of glycosuria and aminoaciduria is no indication of the extent of the general kidney dysfunction. In the chronic form with advanced renal destruction little glucose and aminoacid is excreted, while the young, acutely ill patients excrete the most. We must conclude that the intensity of the glycosuria and aminoaciduria like the polyuria depend more on the severity of the metabolic disorder than on that of the tubular lesion.

(b) **Liver function.** Our patients showed little clinical evidence of liver dysfunction. In the chronic Cases 8, 9, 10 and 11 the liver was slightly enlarged, reaching at most to about one inch below the costal margin, while the spleen remained normal in size. Tests for urobilinogenuria were made frequently but an increase was found only in Case 9. Plasma bilirubin was investigated in Cases 1, 2, 3 and 9, and an increase was found in Case 9 only. The thymol turbidity test was made in eight patients, and a slight increase of 5 and 6.2 units recorded in Cases 1 and 3 respectively. In Cases 1, 2, 8 and 9 the prothrombin time was estimated by a modified McPherson technique, and a slight increase was found in the last two, the highest being 34″ in Case 8 (normal value control 24″). The Takata reaction was carried out only in Case 1 and was negative. The high cholesterol values noted above were attributed to the advanced state of renal insufficiency present in these patients rather than to liver dysfunction. The cholesterol ester in Case 9 was normal (86 per cent).

Abnormalities of the sugar tolerance test and the plasma protein fractions have been discussed elsewhere (pp. 45 and 57). In view of the involvement of the reticulo-endothelial system and of the aminoacid metabolism we do not regard these findings as due necessarily to liver dysfunction alone, nor does it appear likely that the changes in liver parenchyma are responsible for the aminoaciduria in the same way that acute yellow atrophy and other severe liver disorders cause aminoaciduria. Studies in such diseases (ECKHARDT, COOPER, FALOON and DAVIDSON 1948 ; DUNN, AKAWAIE, YEH and MARTIN 1950 ; DENT and WALSHE 1951 ; SCHREIER 1951 ; BICKEL 1952) have shown that much more severe liver destruction than is found in Lignac-Fanconi disease must be present before aminoaciduria of any considerable degree occurs.

C. DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS OF LIGNAC-FANCONI DISEASE

The clinical and chemical features of the disease are shown in Table 10.

Table 10.

DIAGNOSIS OF LIGNAC-FANCONI DISEASE

A. Characteristic clinical findings.
   (a) Dwarfing.
   (b) Cystine storage in eyes and bone-marrow.
   (c) Photophobia.
   (d) Resistant rickets, bony deformities, pathological fractures.

B. Non-specific clinical findings.
   (a) Thirst, polyuria.
   (b) Failure to thrive and to gain weight.
   (c) Anorexia and attacks of vomiting.
   (d) Susceptibility to infection, unexplained fever, metabolic crises.
   (e) Muscular weakness, delayed standing and walking.
   (f) Onset between 6 and 12 months of age, but later in the chronic form.
   (g) Affection of other siblings.
   (h) Latent and manifest tetany in the late stages.

C. Chemical findings (none of which are constant).
   (a) Albuminuria, polyuria, scanty casts and cellular elements in the deposit.
   (b) Variable aminoaciduria and glycosuria.
   (c) Bicarbonate low in plasma, high in urine with alkaline urine.
   (d) Plasma phosphorus low or raised. Phosphatase normal or raised.
   (e) Plasma potassium low, sodium and chloride lowered slightly.
   (f) Cholesterol normal or raised.

In our opinion the presence of Lignac-Fanconi disease should be suspected in all stunted children whose dwarfing is not obviously the result of other causes. Suspicion becomes strongest when there is evidence of kidney dysfunction such as thirst, polyuria, albuminuria and raised blood urea. Other important indications are photophobia, often with sparse fair hair, and the occurrence of similar complaints in siblings. Anorexia, attacks of vomiting and failure to thrive are usual but are not sufficiently specific to be of great diagnostic value.

The chemical findings are so variable that as diagnostic features they are unreliable. This is especially true of glycosuria and hypophosphataemia, and to a lesser degree of aminoaciduria and acidosis, while data about hypopotassaemia are at present only scanty. The testing of body fluids must be repeated several times to be of diagnostic

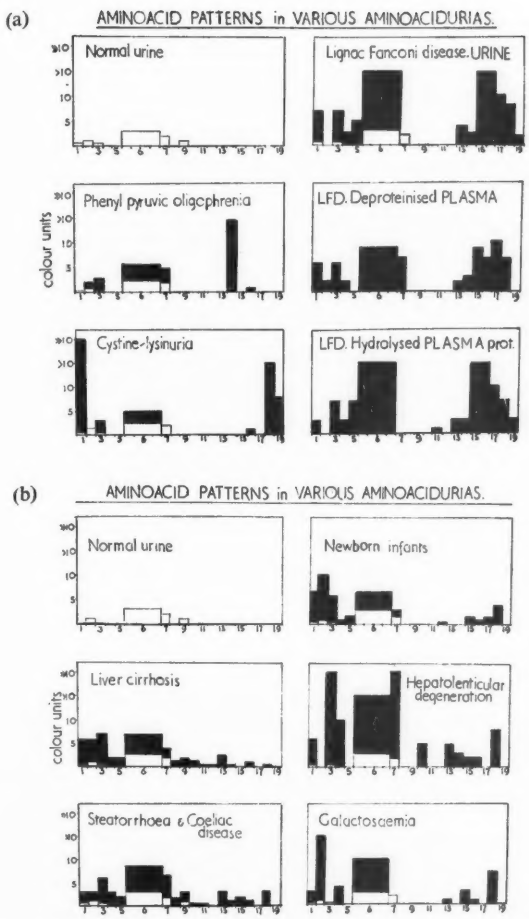


Fig. 10 (a) and (b). The aminoacid pattern of urine chromatograms in eight forms of aminoaciduria, based on the average ninhydrin colour intensity of the aminoacid spots in a limited number of cases of each condition (strictly tentative). Colour units 1 for the weakest. $\geqslant$10 for the strongest colour.

Figures on abscissae : 1 cystine as cysteic acid, 2 taurine, 3 serine, 4 threonine, 5 aspartic acid, 6 glycine + alanine + glutamine + glutamic acid, 7 histidine, 8 methylhistidine, 9 β-amino-iso-butyric acid, 10 γ-amino-butyric acid, 11 methionine as sulfone, 12 ethanolamine, 13 tyrosine, 14 phenylalanine, 15 valine, 16 leucine + isoleucine, 17 proline, 18 lysine, 19 arginine.

value, and sensitive methods such as chromatography must be used to demonstrate aminoaciduria and glycosuria. The aminoaciduria in Lignac-Fanconi disease has a typical pattern which can be differentiated from other forms of aminoaciduria (BICKEL 1952 and Fig. 10).

In the present state of our knowledge we regard the discovery of cystine storage as final proof of Lignac-Fanconi disease. Examination for cystine storage is made either in thick bone-marrow smears or by slit-lamp examination of cornea and conjunctiva. The techniques employed and pitfalls encountered in making these examinations are described in Parts 5 and 8. Here we wish only to warn against the use of any acid or watery dye in the preparation of the bone-marrow smears. Typical crystals are best found at the edges of thick smears and with the aid of polarised light. The search may have to be prolonged.

The most reliable way of detecting cystine storage *in vivo* is probably the microscopical examination of a lymph gland. This was performed recently in Case 1 ; whereas the bone-marrow showed but few cystine crystals, all sections of the gland were full of typical crystals (see Part 8). Chromatography of an extract of this lymph gland showed an excess of cystine. The histological examination of all tissues from the autopsy made on four cases showed a larger number of crystals in lymph nodes than in any other tissue.

The problem of differential diagnosis is summarised in Table 11. Some of the diseases enumerated are easily excluded by careful clinical examination. For example, **benign renal glycosuria, diabetes insipidus** and **acrodynia** do not generally cause dwarfing, have a more benign clinical course and usually a normal chemistry. **Diabetes mellitus** is distinguished by high blood sugar values.

Galactosaemia is characterised by considerable liver enlargement, the presence of cataracts, and by the finding of galactose in urine and blood. In the early stages of the illness without cataract and with only small amounts of galactose in the urine the diagnosis may be difficult. Moreover, in each of three patients with galactosaemia (one case of Dr. SNYDER, New Orleans, personal communication ; two of Drs. BRAY, ISAAC and WATKINS 1952) we found aminoaciduria. The pattern of the aminoaciduria of galactosaemia differs from that of Lignac-Fanconi disease (Fig. 10 and BICKEL 1952).

That **glycogen storage disease** may resemble Lignac-Fanconi disease closely in both its clinical manifestations and chemistry was shown by FANCONI and BICKEL (1949, Case 1). Dwarfing, febrile

Table 11.

Differential diagnosis of Lignac-Fanconi disease.

	Cystine storage	Dwarfing	Rickets	Thirst polyuria	Marasmus	Anorexia vomiting	Hypotonia	Tetany	Albuminuria	Reducing substances in urine	Aminoaciduria	Acidosis	Abnormal phosphorus and / or urea plasma levels
Lignac-Fanconi disease ..	+	+	±	+	+	+	+	±	±	±	+	+	+
Renal rickets	—	+	+	+	+	+	±	±	±	—	—	±	+
Renal dwarfism	—	+	—	+	+	+	±	±	±	—	—	±	+
Resistant rickets	—	+	+	—	—	±	—	—	—	—	—	—	+
Renal acidosis	—	+	±	+	+	+	+	—	±	—	—	+	—
Coeliac disease	—	+	±	—	+	+	±	±	—	—	±[1]	—	±
Glycogen storage disease ..	—	+	—	±	+	+	+	—	—	±	±[2]	±	—
Galactosaemia	—	+	±	—	+	+	+	—	+	+	±[3]	±	±
Diabetes mellitus	—	±	—	+	+	±	±	—	—	+	—	±	—
Benign renal glycosuria ..	—	—	—	—	—	—	—	—	—	+	—	—	—
Diabetes insipidus	—	—	—	+	—	—	—	—	—	—	—	—	—
Acrodynia	—	—	—	—	+	+	+	—	—	—	—	—	—

[1] 3 of 22 patients with coeliac disease were shown by chromatography to have aminoaciduria.
[2] 1 of 3 patients with glycogen storage disease were shown by chromatography to have aminoaciduria.
[3] 3 of 3 patients with galactosaemia were shown by chromatography to have aminoaciduria.
[1] — [3] Bickel 1952.

episodes, acidosis, ketosis, constipation, anorexia, polydipsia, polyuria, renal glycosuria and albuminuria are observed in both diseases, while in the case of glycogen storage disease mentioned above hypophosphataemia with osteoporosis and acidosis were seen. In the same patient chemical and chromatographic examination revealed aminoaciduria, but in its pattern it differed from that of Lignac-Fanconi disease. The principal aminoacids excreted were aspartic acid, glutamic acid, glycine, serine, alanine, histidine and proline, while the aminoacids typical of Lignac-Fanconi disease, namely valine, the leucines, phenylalanine and tyrosine, were lacking. In urine specimens from a second case of glycogen storage disease, sent to us by Dr. C. H. SNYDER of New Orleans, we found no aminoaciduria, and only mild aminoaciduria in a third case referred to us by Dr. BRAID, Birmingham. It may be that the presence or absence of aminoaciduria in this disorder depends upon the extent of the liver and kidney damage which results from glycogen storage. A poor or absent response of the blood sugar to subcutaneous adrenalin injection is a feature of glycogen storage disease in contrast to the well marked sensitivity to adrenalin of Lignac-Fanconi disease (FANCONI and BICKEL 1949). While the aminoacid pattern, the typical doll-like facies of these patients, the more benign clinical course, generally without rickets, and the greatly enlarged liver also help to differentiate glycogen storage disease from Lignac-Fanconi disease, the final proof is the demonstration of massive glycogen storage in liver tissue removed by liver puncture.

Coeliac disease can present a clinical picture indistinguishable from that of Lignac-Fanconi disease and a brief description of such a case, one which remained a problem in diagnosis for a long time, is given here :

R.C. Male. **Coeliac disease with aminoaciduria, dwarfing and photophobia.** From the 4th month this child had anorexia, vomiting attacks, constipation, thirst, irregular temperature, at times as high as 103° F. He disliked bright lights and was unable to stand. At 18 months he was a fair, anaemic, dwarfed, marasmic, hypotonic child with photophobia (Fig. 11). X-ray showed pronounced osteoporosis. Blood chemistry : phosphorus 2.7 mg. per cent, calcium 9.4 mg. per cent, phosphatase 19.7 Jenner-Kay units. Urine : Slight reduction of Benedict's reagent but sugar chromatography was negative. Aminoacid chromatography revealed intermittent aminoaciduria with excretion of glycine, histidine, alanine, glutamine, glutamic acid, serine, alpha-amino-n-butyric acid, taurine and traces of valine, the leucines, phenylalanine and tyrosine. The pattern was different from that seen in Lignac-Fanconi disease. No evidence of cystine storage in bone-marrow or eyes was found. The cornea showed numerous small punched-out areas when stained with fluorescin, indicating an epithelial dystrophy, presumably nutritional in origin and the cause of the photophobia. The diagnosis of coeliac disease was based on the results of a fat balance, which

showed a decreased fat absorption of 88 per cent on a diet containing 50g. of fat, on a barium meal X-ray examination which showed the jejunal loops to be dilated and on a chylomicrograph which showed a subnormal and prolonged rise. On a wheat-free, low-fat diet the child has gained 4 pounds in 2 months and has gone home in much improved health.

Aminoaciduria in children with coeliac disease appears to be rare and although twenty-two other cases have been investigated by chromatography, substantial aminoaciduria has been found in only two

Fig. 11. R.C. and a normal control.

other cases. In chronic steatorrhoea of adults, however, aminoaciduria is more common. A 19 year-old patient of Professor THOMSON and Dr. MACGREGOR showed dwarfing, anorexia, dystrophy, osteoporosis with deformities, steatorrhoea and constant aminoaciduria. In this patient also the diagnosis of Lignac-Fanconi disease was excluded by the atypical pattern of the chromatogram (Fig. 10) and by the absence of cystine storage. The diagnosis of "steatorrhoea" was established by a fat balance. We have examined by chromatography fifty-one further adult patients of Dr. COOKE suffering from idiopathic

steatorrhoea and of these eleven repeatedly showed an excess of amino-acids in the urine (BICKEL 1952).

In **renal acidosis** the history is very similar to that of Lignac-Fanconi disease with vomiting attacks, anorexia, dwarfing, albuminuria, failure to thrive, thirst, etc. Evidence in the plasma of acidosis and an abnormally high pH in the urine due to loss of bicarbonate resemble Lignac-Fanconi disease, but in none of the six patients we have examined has any aminoaciduria or glycosuria been detected. Other distinguishing features of renal acidosis are hyperchloraemia and the normal level of organic acids in the urine.

Resistant rickets, renal rickets, and renal dwarfism are the conditions most difficult to differentiate from Lignac-Fanconi disease. It is probable that in the past cases of Lignac-Fanconi disease were labelled with one of these three diagnoses. Their close resemblance can best be demonstrated by a comparison of the case records of two children recently observed in the Birmingham Children's Hospital.

S.H. **Resistant rickets with hypophosphataemia.**

Female. At 12 months bowing of legs was noticed despite vitamin D prophylaxis. In the following year the deformity increased even with ultra-violet irradiation, appetite became poor, the child was always thirsty, did not gain weight or grow normally. No photophobia. At the age of 6 years she was a small but otherwise healthy-looking child. Weight 36 lbs. Liver and spleen not palpable. Considerable bowing of the legs, some prominence of the costal-chondral junctions and widening of the wrists. B.P. 115/75. X-ray showed active rickets and coarse bone trabeculation. Blood count normal. Blood chemistry : Phosphorus 2.1 mg.%, phosphatase 67 units. Urea, calcium, CO_2-combining power, cholesterol, etc., normal. Urine: pH 6.0, no albuminuria, glycosuria or abnormal cellular elements. Specific gravity from 1005 to 1019. 24-hrs. urine volume normal. Chromatography showed generally normal aminoacid excretion, but on 3 occasions it was at the upper limit of normal with a pattern resembling that of Lignac-Fanconi disease. Ammonia coefficient slightly raised (5.2%), aminoacid coefficient in one estimation normal. Water dilution and concentration test normal, apart from slight hyposthenuria. Urea concentration test normal. Fat absorption good. A mineral balance (Part 7, Table 1) showed decreased calcium and phosphorus retention. This was due to loss of calcium and phosphorus in the faeces, whereas the urine contained diminished amounts of these minerals. A search for cystine crystals in bone-marrow and by slit-lamp examination of cornea and conjunctiva was negative. Progress : under vitamin D in doses of 100.000—200.000 units daily the rickets has healed and the plasma phosphatase and phosphorus have become normal.

Da. Sm. **Renal rickets with uraemia, hyperphosphataemia, hypocalcaemia and hypertension.**

Male. Normal development during the first 2 years then anorexia, failure to gain weight or to grow properly. When ten years old he developed frontal headaches and vomiting, and shortly before admission at the age of 12 yrs. had generalised convulsions. Thirst had always been excessive. On admission,

thin, small, pale boy. height 50", weight 47 lbs. No anaemia, liver and spleen not palpable. No clinical rickets, but X-ray examination showed some decalcification and slight rachitic changes. X-ray of the abdomen showed small kidneys. The renal pelves did not fill at intravenous pyelography. Two opacities suggested stones in the lower end of the right ureter. B.P.230/200. The arteries of the fundi appeared thinner than usual with early silver wiring. Blood chemistry : Urea up to 159 mg. per cent, calcium 8 mg. per cent, phosphorus 8.5 mg. per cent, phosphatase 23 units. Moderate acidosis of 16 mEq/l CO_2-combining power, sodium and chloride level slightly decreased (136 and 92 mEq/l, potassium normal. Cholesterol 168 mg. per cent, albumin/globulin=3.8/1.7 g. per cent. Organic acids (indirect method) increased to 18 mEq/l. Microbiological assay gave normal plasma levels for valine, tyrosine and phenylalanine. Urine : pH 6.6, albumen ++, Benedict and sugar chromatogram negative. No cellular elements. Aminoacid chromatography showed increased excretion of cystine and taurine, slightly increased valine and the leucines, normal glycine, alanine, glutamine and glutamic acid excretion. The ammonia production of the kidney was negligible, and there was an increased excretion of organic acids in the urine (2.4 mEq/Kg/24 hrs., indirect method). The daily urine volume was about 2500 ml., specific gravity 1004. A calcium phosphorus balance (part 7, Tab. 1). was negative for both minerals due to excessive loss in the faeces, whereas their excretion in the urine was considerably decreased. [Slit-lamp investigations for cystine crystals in cornea and conjunctiva was negative, as was a search of bone-marrow smears and sections.

In conclusion, we would stress once more the importance of cystine storage as the distinctive feature of Lignac-Fanconi disease. Only if cystine storage is recognised as an essential finding in the final diagnosis of this disease can a clear-cut line be drawn between Lignac-Fanconi disease and other disorders with aminoaciduria and a similar clinical picture.

D. PROGNOSIS AND TREATMENT OF LIGNAC-FANCONI DISEASE

That the disease has always proved fatal may be deduced from the fact that no case of Lignac-Fanconi disease has been observed beyond the years of puberty. The oldest patient was 16, and few reach their tenth year. In the acute form death occurs early, usually in a metabolic crisis with acidosis and probably hypopotassaemia, which often ensues after an intercurrent infection. In the chronic form the later stages are characterized by progressive kidney destruction. It is possible that this development might be checked by early diagnosis and treatment of the underlying metabolic disturbance. The more advanced this destruction, the smaller will be the chance of successful treatment. However, our own experience suggests that the disease is in fact amenable to treatment and that time may show that a fatal outcome is not inevitable.

The treatment we and our colleagues in other medical centres have used on ten cases in the present series has been largely directed against rickets, acidosis and hypopotassaemia.

Rickets. In some of our patients large and occasionally enormous doses of vitamin D were necessary to achieve absorption of calcium and phosphorus and healing of rickets. Daily doses of between 50,000 and 500,000 units of calciferol by mouth over a period of from 4 to 8 weeks were given to Cases 1, 2, 8, 10 and 11 before the rickets would heal. These doses were well tolerated so long as the X-ray showed active rickets and the plasma phosphatase level remained high. Close supervision of the treatment in hospital is necessary, with daily estimations of urinary calcium, frequent blood pressure readings and at least a weekly check of the plasma calcium, urea, phosphatase and phosphorus levels. The urinary calcium is estimated rapidly by the Sulkowitch bedside test (ALBRIGHT and REIFENSTEIN 1948). With these precautions, the risks of massive vitamin D therapy are few ; here we disagree with FANCONI's recent statement on the subject (1950). A rise in the urinary calcium or in the plasma level are indications to stop vitamin therapy for two to four weeks until the blood and urine levels both become normal, after which the treatment can be continued with smaller doses. As the X-ray appearance becomes normal and the serum phosphatase falls, the daily dose of calciferol should be reduced gradually to about 30,000 units and later perhaps to about 15,000 units. This maintenance dose must be continued for months or years if relapse is to be avoided.

Balance tests (see Part 7, Tables 1 and 2) showed that despite a mixed diet our patients with poor appetites ingest too little calcium and phosphorus. A low phosphorus intake (only 450 mg. daily) was also observed by FANCONI (1936) and one of 470 mg. by MCCUNE, MASON and CLARKE (1943), while the calcium intake was 434 mg. and 745 mg. respectively. As well as large doses of vitamin, we have given one gram of calcium phosphate daily with the food.

That vitamin D may improve the condition of the bone even in the last stages of low calcium—high phosphorus rickets is shown by PHILPOTT's second case (see Part 6). In such cases, however, the combination of hypocalcaemia and acidosis constitute a difficult therapeutic problem. Alkalinisation may cause tetanic attacks and should be employed only after the plasma calcium level has been raised. This is not easy to achieve with calcium by mouth ; in Case 9 the blood calcium remained at 6 mg. per cent despite large doses of vitamin D and calcium

by mouth. A rise was achieved only by giving calcium intravenously, a treatment that could hardly be maintained over a long period.

Acidosis is best relieved by the use of Albright's solution. We have prescribed :

Sod. citrate .. 100 grams
Citric acid .. 140 grams
Distilled water to 1,000 ml.

in doses of between 10 and 50 ml. five times daily according to the severity of the disturbance. Adequate alkalinisation of these patients has its dangers and a small increase in the daily dose may induce alkalosis in the early stages of treatment when metabolism is still unstable. We advise a small initial dose of alkali, gradually increased and controlled by frequent testing (often 2 or 3 times weekly) of the CO_2-combining power in the plasma, until the desired result has been reached. A normalisation of the CO_2-combining power between 25 and 29 mEg/l. was achieved in all patients treated by us and could be kept within this range for months. The citrate mixture was never refused by these extremely thirsty children.

Hypopotassaemia. It was only recently that we discovered the importance of hypopotassaemia in this disease, and thus our experience of its treatment is limited to twelve months. When the child's hydration is good we use half a gram of potassium chloride twice daily by mouth and, after checking the plasma potassium level, make increases up to 1 gram twice daily. If the chloride is refused because of its unpleasant taste, potassium citrate can be used to replace some of the sodium citrate in Albright's solution, e.g.

Pot. citrate .. 50 grams
Sod. citrate .. 50 grams
Citric acid .. 140 grams
Distilled water to 1,000 ml.

giving 15 ml. five times daily by mouth. If the dose of alkali is increased, then the percentage of the potassium salt in the mixture should be reduced to avoid over-dosing with potassium.

Metabolic crises. These characterise the acute form and are often relieved only by intravenous infusion therapy, the results being usually no less dramatic and satisfactory than those of treating severe diabetic coma. What might be termed the basic infusion is dextrose and physiological salt solution, with occasional blood, plasma or casydrol. The more specific form of treatment of the crisis must be related to the

blood chemistry. Intravenous alkali is nearly always necessary and also potassium which may be given in the form of Butler's solution, but only after water repletion of a seriously dehydrated child. In the presence of severe vomiting infusions of hypertonic sodium chloride solution may be indicated. With intensive treatment recovery from a crisis occurs usually quite rapidly with still further improvement in the general condition if such treatment is maintained.

Other therapeutic measures. Occasional blood transfusions were given to five of our patients and appeared to have a beneficial effect in increasing energy and appetite. Children with anaemia and low gastric acidity were given ferrous sulphate gr. 3, acid hydrochlor. dil. m. 5, three times daily shortly before meals, but since systematic alkali and potassium therapy has been established these agents have become unnecessary. The increased urinary excretion of sodium chloride indicates the importance of an adequate salt intake. In patients with polyuria a large intake of fluid by mouth is essential in order to replace the enormous fluid loss in the urine (as much as five litres per day). The extreme anorexia for solid food which characterises the acute form of the disease precludes the use of special diets, but because of thirst our patients were given plenty of sweetened citrated milk. No detailed account is given here of the numerous therapeutic trials made on our patients without obvious success. They include the use of adrenal cortical hormones, pitressin, testosterone, choline, vitamin B complex, vitamin C, injections of liver extract, daily plasma transfusions, casein hydrolysate, etc.

In five patients we have observed the effects of combined therapy for acidosis, hypopotassaemia and rickets over a period of months, and there is no doubt that it has had an important influence on the course of the disease. CO_2-combining power and potassium levels in the plasma are now normal. Dangerous acidotic crises are no longer experienced and recovery from measles and other intercurrent infections has been uneventful. Appetite has improved considerably, height and weight are slowly increasing. Anaemia has disappeared. Vomiting is now unusual or has ceased entirely, especially since potassium therapy has been instituted. The children are more active, less hypotonic and three are now walking for the first time (Fig. 12).

With vitamin D treatment rickets has healed slowly and osteoporosis has regressed so that we hope to have prevented the development of the crippling deformities which have characterised older children with Lignac-Fanconi disease (Cases 9 and 11 of this series) often confining them to bed for years, though they may have seemed

otherwise fairly well. Perhaps the most striking and prognostically the most important effect of prolonged alkalinisation has been the gradual reduction and eventual disappearance of aminoaciduria and glycosuria. We do not claim that this therapy corrects all the disturbances of Lignac-Fanconi disease nor do we know whether

Fig. 12.
Case 2 showing improvement under treatment. (Nov. 1951).

or not it will finally prevent a fatal outcome. The improvement achieved has not included really satisfactory growth or normal gain in weight. Thirst and polyuria remain unchanged, while energy and appetite, though improved, are variable. Nevertheless, by comparison with their condition before treatment, our patients show such obvious improvement that we cannot avoid an attitude of cautious optimism.

SUMMARY

Clinical manifestations of Lignac-Fanconi disease are dwarfing and wasting, rickets and osteoporosis, pyrexia, eye changes, sometimes with photophobia, vomiting, polydipsia and polyuria, dehydration, acidosis, sometimes tetany, states of profound collapse and even sudden death. Acute and chronic forms of the disorder are described.

Laboratory investigations reveal aminoaciduria, glycosuria, and sometimes albuminuria and ketonuria. In the plasma there is amino-acidaemia, evidence of acidosis, hypopotassaemia, mineral changes as seen in rickets, and perhaps hypocalcaemia and uraemia, which may be renal or extrarenal.

In a short factual analysis the phosphorus-calcium, protein, electrolyte and sugar metabolism as well as liver and kidney functions are discussed. A special section deals with E.C.G. changes, and electrophoretic investigations are described in an addendum.

The diagnosis "Lignac-Fanconi disease" should be suspected in all stunted children whose dwarfing is not obviously the result of other causes. Rickets, sometimes with severe deformity, evidence of kidney dysfunction and glycosuria are further clinical features, while the discovery of cystine storage and the demonstration of aminoaciduria with a characteristic aminoacid pattern provide final proof of Lignac-Fanconi disease. Differential diagnosis from renal rickets, resistant rickets, coeliac disease, renal acidosis, galactosaemia and glycogen storage disease may sometimes be difficult.

Treatment of the rickets with massive vitamin D doses, of the acidosis with Albright's solution and of the hypopotassaemia with potassium salts has proved encouraging; acidotic and hypopotassaemic crises have ceased, the children gain weight and grow slowly, their rickets is cured and they are livelier. Perhaps the most striking effect of prolonged alkalinisation is the gradual disappearance of amino-aciduria.

The case records are given in an addendum.

ADDENDUM 1

PROTEIN ANALYSES IN LIGNAC-FANCONI DISEASE

by

J. HARDWICKE* and **D. R. STANWORTH***

from

THE DEPARTMENT OF EXPERIMENTAL PATHOLOGY,

UNIVERSITY OF BIRMINGHAM

A number of serum protein analyses were carried out on three cases of Lignac-Fanconi disease, and on two normal children, by the method of paper electrophoresis (DURRUM 1950, CREMER and TISELIUS 1950, TURBA and ENENKEL 1950, KUNKEL and TISELIUS 1951, GRASSMANN, HANNIG and KNEDEL 1951) using a modification of the method described by TURBA and ENENKEL.

Strips of Whatman No. 2 paper, 6 cm. × 38 cm., were moistened with N/20 barbiturate buffer pH 8.6, and five drops of serum (diluted if necessary with buffer to give a final total protein concentration of 2—5 g.%) were applied at equal intervals over the centre 2 cm. of a line drawn across the paper. The strips were then enclosed in a "perspex" box, 23 cm. × 10 cm. × 5 cm., so that the applied protein lay 4—5 cm. from one end. The free ends of the paper were dipped into two vessels containing buffer, the liquid levels being balanced and connected to two electrode vessels by a bridge of 0.5% agar in buffer. A current of one milliamp was passed for 17 hours—the total length of paper between the vessels was 32 cm. After drying, the strips were stained for 30 minutes in 0.1% brom-phenol-blue in ethanol saturated with mercuric chloride (Durrum 1950), fixed vertically in a frame, and washed in tap water for 30 minutes (Birmingham tap water has a pH of 7.0 and specific conductivity at 0° C. of 5.0×10^{-4} mhos/cm.), a steady flow being maintained by a constant head device. After drying, the relative concentrations of the protein fractions were determined by cutting the paper into segments, eluting the dye from each segment with 7 ml. 2.5% sodium carbonate and determining the optical density of each eluate against a blank for protein free paper in a Hilger "Spekker" using a yellow-green filter, Ilford No. 606. Duplicate determinations were made on two separate 17-hour runs. This method shows good reproducibility, with values for the different fractions agreeing substantially with those obtained by free-electrophoresis in the Tiselius apparatus using N/10 barbiturate buffer at pH 8.6 (Longsworth 1942), the values by the paper method being rather higher for globulin, and lower for albumin.

Table I shows the comparison of the two methods in a normal child, and in one with Lignac-Fanconi disease.

Approximate total protein values were obtained from the serum specific gravity determined by the copper sulphate method of Van

* In receipt of grants from Medical Research Council.

Table I

Case	Method	Protein %				
		Albumin	α_1	α_2	β	γ
Normal ..	Tiselius (mean of asc. and desc.)	54.9	4.8	11.5	14.5	14.3
	Paper (mean of 2)	39.6	6.0	17.5	15.8	21.1
Fanconi ..	Tiselius (mean)	49.2	6.3	13.1	15.4	16.4
	Paper	44.4	6.0	17.4	12.2	20.0

Slyke (PHILLIPS, VAN SLYKE et al. 1945). Fibrinogen values were obtained as "clottable" nitrogen by the technique of Van Slyke (PETERS and VAN SLYKE 1946).

Table II shows the values obtained in the cases studied.

Table II

Case	Total Protein (g%)	Fibrinogen (g%)	Albumin	Protein % α_1 glob.	α_2 glob.	βglob.	O	γglob.
1. Normal child aet. 4	6.1	—	Serum 57.1	3.0	11.4	9.6	—	18.9
			Serum 58.5	4.6	7.7	8.8	—	20.4
2. Normal child aet. 3½	6.1	—	Serum 42.3	6.2	15.4	16.6	—	19.5
			Serum 36.9	5.8	19.7	14.9	—	22.7
3. Normal child (repeat 2) aet. 3½	6.8	0.360	Plasma 49.5	6.5	14.9	11.0	5.0	13.1
			Serum 46.8	6.0	14.7	13.8	—	18.7
4. Case 2 (1)	7.4	0.400	Serum 60.1	4.3	12.2	12.7	—	10.7
5. Case 2 (2)	5.3	—	Serum 52.2	6.6	15.0	13.3	—	12.9
6. Case 8 (1)	6.8	—	Serum 44.4	6.0	17.4	12.2	—	20.0
7. Case 8 (2)	7.5	—	Serum 46.7	5.6	14.4	12.4	—	14.9
8. Case 1	6.7	—	Serum 45.1	4.9	19.8	11.6	—	18.6

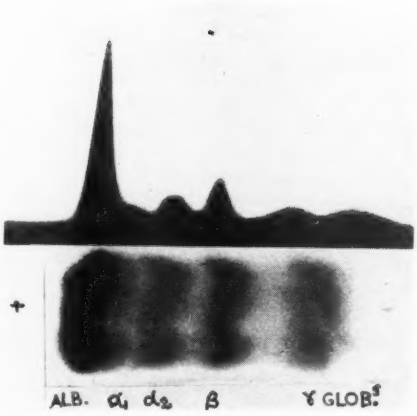


Fig. 1.
Normal electrophoresis findings.

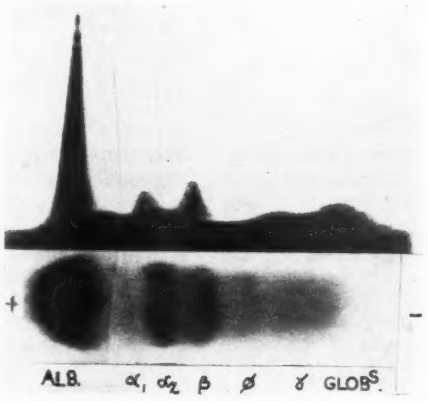


Fig. 2.
Electrophoresis findings in Case 2.

Figures 1 and 2 show the schlieren diagram on free electrophoresis compared with the paper strip obtained for Normal Child (No. 2) and Case 2 (No. 4). In Figure 2 the paper strip run is of plasma, and the extra component, with no counterpart in the Tiselius, can be seen between the γ—globulin and the β—globulin.

The figures are insufficient for statistical analysis, in view of the small numbers and considerable variation in the normal children, but no gross deviation in the diseased children has been shown.

In both groups the α_2—globulin values are higher than those usually found in normal adults, where any value over 12-14% of a total serum protein of 6.0-6.50 g.% is abnormal.

ADDENDUM 2. CASE RECORDS

NOTE

A detailed account of the numerous biochemical and haematological findings in Cases 1-9 has been omitted in order to shorten the case records.

Some figures can be found in this part in Tables 1-8.

For chromatographic, microbiological and glutamine estimations, see Part 7, Tables 5—8.

For calcium-phosphorus balances, see Part 7, Table 1, and for electrolyte values see Part 7, Tables 9—12.

For records of Cases 10 and 11, see PHILPOTT, HARVEY and FINCH, Part 6.

Cases 15 and 16, mentioned in Part 8, are being published by Dr. R. J. K. BROWN (1952). A detailed necropsy report will be given in Part 8.

CLINICAL RECORD OF CASE 1

K.C., male, born 5.9.48, age on admission 1 yr. 3 mths. Lignac-Fanconi disease and rickets. Acute form, first seen in an early stage. Great improvement with treatment.

Family History. No consanguinity of parents. The patient's mother had glycosuria during pregnancy. Two maternal siblings died "at an early age," one of "gastro-enteritis", the other of "vomiting." A paternal great-aunt was dwarfed and died of "drinking diabetes" at the age of 19 years. The paternal grandmother has diabetes mellitus but no aminoaciduria. Thirteen further members of the family have been tested by chromatography for aminoaciduria and glycosuria with negative results.

Case History. Quite healthy for first 6 months, while breast-fed. Then given mixed diet of meat, vegetables, etc. After weaning ceased to thrive and stopped growing. Poor appetite, attacks of severe vomiting lasting several days, abnormal thirst—drinking up to 4 pints a day—polyuria with incontinence and constipation. Weight at 6 months 14 lbs., at 1 year 16 lbs. 3 oz. with no further increase in the next 9 months. One teaspoonful of cod liver oil daily since birth. Admitted to the Children's Hospital, Birmingham, 19.12.49, after vomiting almost all food and drink for 10 days.

Admission Findings. Weight at 15 months 16 lbs. 12 oz., height 28 ins., proportions normal, subcutaneous tissue and musculature moderate, hypotonia well marked (Fig. 1). Unable to stand. Extremities almost always cold. Teeth normally developed and healthy. Bone ends enlarged, rosary present, no other bony deformity, fontanelle almost closed. Liver and spleen not enlarged. Hair colouring medium brown. No photophobia. BP 105/65. X-ray showed active rickets and slight decrease in bone density. Bone marrow showed a few cystine crystals. Slit-lamp examination revealed crystalline deposits in cornea and conjunctiva.

Chemical findings and haematological data. The most important findings were the low plasma phosphorus, the high phosphatase and the normal urea and calcium levels. In the balance experiments the phosphorus and calcium loss was shown to occur in the faeces. Of 14 potassium estimations by flame photometer (Dr. Barclay) there was on 12 occasions a hypopotassaemia sometimes as low as 2.1 mEq/l. A potassium balance on a mixed and complete toddler diet

during a period of clinical well-being was positive. On an average daily intake of 3407 mg., 451 mg. were retained ; 90% of the total potassium excretion was found in the urine. A nitrogen balance under similar conditions was positive, 98% of the intake being absorbed and 33% retained ; 97% of the total nitrogen excreted was in the urine. A second nitrogen balance gave a similar result, 95% of the intake being absorbed and 15% retained ; 95% of the total nitrogen excreted was in the urine. Without alkali the child was acidosed but was able to form increased amounts of ammonia in the kidney. During acidosis abnormal amounts of bicarbonate were often found in the urine. The pH of the urine did not fall below 6 except in an acid-feeding experiment when it dropped to 5.3. Massive and constant amino-aciduria, with an excretion of 15 or more aminoacids, was accompanied by raised levels of various aminoacids in the plasma. Glycosuria, which was constant, was shown to be renal. Glucose was identified by chromatography. A glucose tolerance test after 15 g. glucose orally showed blood levels :—fasting 80 mg., 30 mins. 127 mg., 60 mins. 135 mg., 90 mins. 110 mg., 120 mins. 106 mg., 150 mins. 99 mg.%. Glycosuria was present with a blood level of 80 mg.%. Ketonuria was sometimes present. Casts, pus cells and traces of albumen were occasionally seen in the urine. The daily urine volume was increased up to 3200 cc. and the specific gravity was fixed between 1010-1012. Liver function tests showed thymol turbidity 5 units, serum bilirubin 0.2 mg.%, Takata negative, fibrinogen 0.5g.%, no increased urobilinogenuria. In a fat balance only 82% of a 30 g. fat intake was absorbed. To a 50 g. fat intake the child responded with vomiting and loose stools. The haematological data were essentially normal. ECG : G p Tc long, 0.435 sec. T and T_2 flat topped. A definite change.

Course of the disease and treatment. The child's general condition varied rapidly from one of apparent well-being to one of anorexia, dehydration, drowsiness, collapse and acidosis often associated with attacks of severe vomiting. Hypo-potassaemia was found later but was not investigated during these attacks, in which it may have played an important part. Deterioration in the child's condition was frequently preceded by some minor infection. On three occasions subcutaneous or intravenous infusion therapy was given. Further difficulties arose after treatment for rickets and acidosis was instituted. A daily dose of 100,000 units of calciferol over a period of four weeks proved inadequate. The dose was doubled and after two weeks partial healing took place but toxic signs such as vomiting and anorexia, which were attributed to vitamin D intoxication, developed, and the treatment with vitamin was discontinued. Similarly, alkali therapy was either ineffective in relieving the acidosis or, when the dosage was increased slightly, caused alkalosis. Thus, within a few days of increasing the intake of sodium and potassium citrate from 13 to 16 grams daily by mouth the CO_2-combining power of the plasma rose from 19.8—38.7 in mEq/l., and convulsions occurred. Alkali treatment was stopped immediately and the CO_2 value dropped to 6.8 mEq/l. During the last year better alkalinisation has been effected by using a mixture of sodium citrate 100 g. and citric acid 140 g. in one litre of water in a dose of 25 ml. by mouth 5 times daily. When by this means the CO_2-combining power of the blood remained within normal limits for some weeks the aminoaciduria and glycosuria decreased and eventually disappeared, reappearing when the blood again showed evidence of acidosis. This change in the character of the urine was especially striking, since before alkali therapy this child had shown a strong and persistent aminoaciduria, which had not been influenced by other remedies such as heparglandol, testosterone, eschatin,

insulin, riboflavin, nicotinamide, yeast, vit. D, casydrol or blood transfusion. After 2 years hospitalisation the child has now gone home. He has learnt to stand and to walk, has gained 4 lbs. 7 oz. and has grown 2 inches. His mental development seems somewhat retarded.

CLINICAL RECORD OF CASE 2

P.R., female, born 28.6.48, age on admission 1 yr. 4 mths. Lignac-Fanconi disease with active rickets, acidotic crises. Acute form, grave constitutional disturbance. Considerably improved by treatment.

Family History. No parental consanguinity. Patient's mother had a "diseased hip" in early childhood. A maternal cousin and two aunts had "kidney trouble" and the maternal grandfather had "rare kidney stones" from early youth. A maternal great-aunt had Bright's disease, while a paternal great-aunt is said to have had diabetes. Eighteen members of the family have been tested by chromatography for aminoaciduria and glycosuria with negative results.

Case History. Breast fed for 9 months and then received adequate mixed diet with plenty of vegetables and fruit. During weaning she started to vomit and lost about 2 oz. in weight weekly. Became rather constipated and developed abnormal thirst and polyuria. Examination revealed sugar in the urine and the child was referred to the Children's Hospital, Birmingham, on 5.10.49, as a case of "diabetes" and admitted.

Admission Findings. Weight at 1 yr. 4 mths. 18 lbs. 2 oz., height 29 ins. A pale and ill-looking child, small, dystrophic and dehydrated (see Fig. 1) with very sparse fair hair but without photophobia. Refused food, drank with avidity and vomited frequently. Bone ends enlarged and rosary present but no other deformities. Teeth discoloured and lower incisors fluted. Three decayed teeth had to be extracted. Intelligence normal but remained shy and moody. Extremities tended to be cold and cyanotic. No oedema. B.P. 85/55. Muscles hypotonic, could not stand and showed no inclination to sit up. X-ray showed moderate rickets with marked decrease in bone density generally. There were no fractures or other bony changes. Bone marrow showed a moderate amount of cystine crystals (see Part 8). Slit-lamp investigation showed diffuse crystalline deposits in the cornea and conjunctiva.

Chemical findings and haematological data. Summarising the most important findings, the urea varied from normal to slightly raised levels, the phosphorus levels were generally low, though normal and, on two occasions, high levels were found under vitamin D therapy. This treatment also caused a return to normal of the slightly raised phosphatase levels. Serum calcium levels were usually normal but occasionally slightly raised during treatment with vitamin D. In a 24-hr. collection of urine the excretion of calcium was decreased while the phosphorus excretion was on the upper limit of normal. Before treatment with alkali the child was acidosed but survived a number of severe acidotic crises. The pH of the urine nevertheless remained above 6.7 and bicarbonates were lost in the urine in abnormal amounts. The ammonia production by the kidney was normal or slightly raised. In the urine there was a greatly increased excretion of 15 or more aminoacids, and their level in the plasma was also raised, as shown by microbiological assay and by chromatography. Glycosuria was well marked but inconstant. The sugar was identified by chromatography as glucose. Sugar tolerance test : morning fasting urine specimen 0.25 g.%, blood sugar 100 mg.%. After 15 glucose orally : 30 mins. blood sugar 220 mg.% ; 60 mins. 114 mg.%

and urine sugar 2 g.% ; 90 mins. blood sugar 113 mg.% and urine sugar 2 g.% ; 120 mins. blood sugar 102 mg.% and 150 mins. 91 mg.%. In four further tolerance tests the influence of the sugar ingestion on the potassium level in the plasma and the CO_2-combining power in blood were studied. Fig. 2, Part 7, shows that during the sugar test the potassium level dropped as low as 2.9 mEq/l so that the shocks which have been described during sugar tolerance tests in this disease may well have been due to hypopotassaemia. The CO_2-combining power was not influenced by the test. Further estimations of plasma potassium were carried out during the later months of the investigation, when marked clinical improvement had followed alkali therapy. Of 14 potassium estimations on fasting plasma 9 specimens showed potassium levels of under 3.8 mEq/l.

Pus cells and albuminuria were found occasionally. Daily urine volume always high, sometimes up to 3 litres ; specific gravity ranged from 1003-1015. There was at times a marked normochromic anaemia with a haemoglobin of 7.4 g.%, R.B.C. 2.9 millions per cmm., as well as leucocytosis up to 24,000 with neutrophilia. Thymol turbidity 2 units, total cholesterol 248 mg.%, free cholesterol 80 mg.%, serum bilirubin 0.4 mg.%, urobilinogenuria not increased. Fibrinogen 0.40 to 0.67g.%. Duodenal intubation revealed trypsin and amylase in normal concentrations. A 3-days' collection of stool showed 24.5% total fat in the dried faeces. E.C.G. : T taller but bifid in lead 1 and 2 ; biphasic or inverted in chest leads. S-T depression in all leads. Very long R-T (.525 sec.). A marked change suggestive of hypopotassaemia.

Course of the disease and treatment. During her first five months in hospital she remained ill with frequent attacks of vomiting, periods of high temperature, severe anorexia, dehydration and great prostration. These crises were commonly associated with severe acidosis. She sat up with reluctance and movement appeared to be a painful effort. At that time the serum potassium was not checked but may have been an important factor. On four occasions intravenous infusions with alkali, salts, plasma and casydrol were necessary. Twice the blood sugar rose to values of 225 and 200 mg.%, whereas the level of urea in the plasma was only once above 100 mg.%. During the crises the aminoacid levels in urine and plasma were little changed but dropped when intravenous alkali therapy was started. After five months in hospital, alkalinisation was commenced with a mixture of sod. citrate 100 grams, citric acid 140 grams, water to 1000 ml. in doses of 20 to 25 ml. 5 or 6 times daily by mouth. With long continued alkalinisation the vomiting attacks have become less frequent ; metabolic crises and fever have disappeared. Amino-aciduria and glycosuria gradually diminished and finally ceased. When alkali therapy was temporarily stopped it was some weeks before the aminoaciduria reappeared. Appetite has returned and the child has slowly gained weight ; she now weighs 4 lbs. more than on admission $1\frac{3}{4}$ years ago. During this time she has grown only 1 inch. The rickets remained unhealed until the daily calciferol dose was increased from 100,000 units daily (for 10 days) to 500,000 units (for 6 weeks). It was then reduced for 4 weeks to 250,000 units daily by mouth. On this high dosage the rickets was cured completely (see Part 5) and has remained healed with daily doses of at first 30,000 and later 10,000 units of calciferol by mouth. The hypopotassaemia is now being treated with potassium chloride, 2 grams daily, which has reduced the incidence of vomiting still further. On this treatment the child returned home, where she continues to make good progress. At the age of $3\frac{1}{2}$ years she has now learnt to walk (Fig. 12).

ABSTRACT OF THE CLINICAL FINDINGS IN CASE 3

M.G., female, age on admission 1 yr. 6 mths. Lignac-Fanconi disease with osteoporosis but no rickets. Acute form. Collapse during sugar tolerance test.

We are indebted to Professor Fanconi for permission to publish a summary of this case which has been recorded in detail by FANCONI and BICKEL (1949).

Family and Case History. The patient was 18 months old when admitted to the Children's Hospital, Zürich. Her only sibling, a brother, died at 3 years 6 months of cystine storage disease. One miscarriage followed the birth of the son. No other related disease in the family. No consanguinity. The child was healthy up to the age of 12 months, but after a febrile illness with bronchitis she refused food, lost weight and developed continuous thirst and frequent attacks of fever. Examination of the urine on several occasions revealed albumen and sugar.

Admission findings. Her height and weight were those of a 10 months old baby, though her mental development appeared normal. Skin and mucous membranes were pale and she appeared ill and dehydrated (Fig. 1). No evidence of rickets on clinical or X-ray examination. Her fontanelle, however, was still wide open and there was a moderate but generalised osteoporosis. The child had received one large dose of vitamin D (600,000 units) at the beginning of her second year. Teeth normal, muscles hypotonic. Liver and spleen not enlarged. B.P. normal. Cystine crystals were seen in the bone marrow but not in cornea or conjunctiva, perhaps because the slit-lamp investigation was hindered by the child's restlessness.

Summary of haematological and biochemical findings. Normochromic anaemia (Hb. 9.8g.%) with decreased erythropoesis and moderate neutrophilia revealed by marrow puncture. Blood chemistry included normal N.P.N., calcium and phosphatase values and low phosphorus. The alkali reserve was always decreased and the urinary ammonia excretion raised, with pH ranging from 5 to 7.8. The plasma cholesterol was increased. Electrophoretic investigations showed an increase of the alpha and beta globulins and a decrease of albumin. Thymol turbidity once increased to 6.2 units, cadmium reaction twice positive. Bilirubin normal. In the urine an increased excretion of various aminoacids was demonstrated by paper chromatography and by formol titration. Reducing substances were detected occasionally and identified as glucose. No ketonuria. A sugar tolerance test showed the glycosuria to be renal. In the course of a second tolerance test when 3 doses each of 20 grams dextrose were given at hourly intervals the third dose was vomited, the pulse became weak and accelerated to 160/min. and the child collapsed, but soon recovered. The blood sugar was then 180 mg.% and there was glycosuria but no ketonuria. Unfortunately no potassium estimations were carried out, but an E.C.G. on another day showed a flat two-peaked T in lead II as well as in the chest leads. It is possible, therefore, that hypopotassaemia was present and that it played an important part in the collapse.

The urine volume appeared to be normal in two 24-hour collections. The mother stated that at home the child was always wet. Water dilution and concentration tests showed significantly delayed excretion of the water ingested. The specific gravity ranged from 1001 to 1008. Albumen up to 0.2 g. per cent was found in all but one urine specimen. The centrifuged deposit contained a few pus cells only.

Course of the disease. During her 3 weeks in hospital she remained very ill, vomiting frequently and for no obvious reason. Her temperature was occasionally raised to 105° F, and sulphonamides and penicillin had no effect. She did not gain weight and her condition when she was finally sent home was unchanged.

CLINICAL REPORT OF CASE 4

R.C., male, born 24.6.45, age on admission 1 yr. 4 mths. Lignac-Fanconi disease without rickets. Atypical chemical findings. Severe constitutional disturbance. Death at 3 years from respiratory infection.

Because of the atypical clinical picture the correct diagnosis was established only at autopsy. The clinical investigation was therefore incomplete and no attempt was made to show aminoaciduria.

Family History. No parental consanguinity. Three siblings alive and well. One miscarriage after this child. Parents healthy. A paternal aunt died of uraemia at the age of 13. She was small with rickets and the autopsy revealed extremely small, pale and markedly granular kidneys. Two further relatives of the father had suffered from kidney disorders. Five members of the family have been tested by chromatography for aminoaciduria and glycosuria with negative result, but the father, a paternal uncle and cousin showed typical cystine-lysinuria (see footnote Part 1, p. 19).

Case History. Breast fed for the first 6 months, then Robinson's Groats and Benger's food, but did not take to solids. No fruit, but cod liver oil and orange juice from birth for the first two years and then Adexolin. Sat up at 7 months, first tooth at 11 months. Never walked. Developed normally up to six months, then vomited severely for 3 days. Subsequently these vomiting attacks recurred every week or two. Lost weight from 16½ lbs. at 6 months to 14 lbs. 8 oz. at 16 months. Refused all solid food, took only fluids and was rather constipated. Rickets had been diagnosed at 6 months by X-ray investigation, but the films could not be traced. The child was admitted to the Children's Hospital, Birmingham, on November 12th, 1946.

Admission Findings. Weight at 16 months 14 lbs. 8 oz., height 26½ inches. A pale, flabby, wasted child with a protruding abdomen and lax, dehydrated skin, apart from the limbs, which showed oedema. Fontanelle wide open but no other clinical signs of rickets. His five teeth seemed good. Photophobia was not mentioned, and no slit-lamp investigation or bone marrow puncture was performed. Liver and spleen were not palpable. C.N.S. and intelligence appeared normal. X-ray showed normal bones without signs of osteoporosis or rickets. The carpal ossification was rather delayed. An intravenous pyelogram showed concentration in both kidney pelves to be poor, but no pelvic enlargement was seen.

Chemical findings and haematological data. To summarise the findings, the urea in the plasma was normal in one test but was increased in all other estimations, and rose to 300 mg.% shortly before death. The one serum calcium estimation after admission was normal ; the alkaline phosphatase was slightly raised as was the serum phosphorus (6.2 and 6.5 mg.% shortly before death). No investigations of the acid base or aminoacid metabolism were carried out. In the urine there was occasionally a slight albuminuria with a few pus cells and red cells in the centrifuged deposit. Reducing substances and ketone bodies were absent on repeated testing. Daily urine volume was not measured. Blood count showed leucocytosis with neutrophilia or lymphocytosis but no anaemia. A 6-day collection of stool showed a total fat content of 29.4 % of the dried faeces on a "toddler" diet. A provisional diagnosis of renal dwarfism with severe renal failure was made.

Course of the disease. Shortly after admission vomiting and dehydration became so severe that an intravenous infusion of glucose and saline was given. He was febrile up to 104° F. for no obvious reason. He survived this crisis, but gained no

weight and after 5 weeks in hospital was sent home with the diagnosis of renal dwarfism and a poor prognosis. Eighteen months later, when nearly 3 years old, he contracted an upper respiratory tract infection, as did several other children in the house. Sulphonamides had no effect and a few days later he was admitted to Dudley Road Hospital, Birmingham, pale, semi-conscious, restless, with generalised oedema and a dry, furred tongue. Dwarfing was noted but he showed no evidence of rickets on clinical examination. There was generalised slight abdominal tenderness. Chest examination normal. B.P. 65/25. Blood count Hb 6.2g. %, RBC 2. 28 mill., WBC 39,000, of which 69% were polymorphs and 27% lymphocytes. Blood urea 300 mg.%, phosphorus 6.5 mg.%. Urine : an occasional RBC, no casts, culture sterile. Stools : culture sterile. The child's condition deteriorated very rapidly, despite intravenous infusions of glucose and saline, and he died on June 13th, 1948.

Autopsy findings. Dilation of the right ventricle of the heart, extremely pale splenic pulp and contracted kidneys with yellow linear marking in cortex and pyramids. Histological examination showed chronic interstitial nephritis and widespread cystine deposits throughout the body (for details see Part 8).

Our thanks are due to Drs. G. W. HEARN and WHITELAW of Dudley Road Hospital for their account of the clinical and post-mortem findings, for their gift of paraffin blocks of various organs and for permission to publish the relevant data.

CLINICAL RECORD OF CASE 5

J.N., male, born 11.10.47, age on admission 2 yrs. 6 mths. Lignac-Fanconi disease without rickets. Subacute form, constitutional disturbance rather severe. Considerable improvement under therapy.

Family History. A healthy family without consanguinity. One miscarriage before this child. A younger sister aged nine months appears healthy. Eleven members of the family have been tested by chromatography for aminoaciduria and glycosuria with negative results. The mother had lactosuria during pregnancy.

Case History. For the first 9 months he appeared quite normal, except for some feeding difficulties after weaning. At 10 months his appetite diminished and he lost weight. His first word was "drink." At 14 months sugar was found in the urine. At 15 months he vomited all food during a stay of 10 days in a hospital and lost 4 lbs. There was some improvement after citric acid therapy. At 17 months, after blood chemistry investigations and the demonstration of aminoaciduria, the diagnosis "De Toni-Debré-Fanconi syndrome" was made, although there was no rickets or osteoporosis to be seen in the X-ray. Vitamin D therapy in the form of 10 drops of concentrated Adexolin daily was commenced. He had had cod liver oil from birth up to sixteen months. At 2 years vomiting ceased and his appetite improved slightly but he was a very "finicky" eater (mushrooms for breakfast), with a tremendous thirst, drinking about 2 quarts in the 24 hours and waking almost every hour of the night to drink. With an attack of influenza, thirst disappeared completely but temporarily. Growth appeared to cease after one year and his weight never exceeded 20 lbs. He could not walk until he was two years old, never ran and disliked climbing stairs. Photophobia was first observed during his second summer, when he appeared disinclined to play in the sunshine, wanted the curtains drawn and was dazzled by electric light. He was never drowsy, had no tetany, no fractures or deformities and no temperature. He was admitted to the Children's Hospital, Birmingham, on March 14th, 1950. Chemical findings prior to this admission

included : at 14 months sugar in the urine ; at 17 months plasma CO_2 17.3 mEq./l, NaCl 590 mg.%, urea 30 mg.%, phosphorus 3.4 mg.%, calcium 11.2 mg.%, alkaline phosphatase 18.4 units, cholesterol 109 mg.%, blood sugar 85 mg.%. The specific gravity of the urine was 1001 and traces of albumen, sugar and acetone were present, but no abnormal cellular elements.

Admission Findings. Weight at 2½ years 20 lbs., height 32 inches. A delicate wasted child, with pale skin, fair hair, and marked photophobia (Fig. 1). He was always thirsty and dripped urine continuously. The extremities were cold and cyanotic, the musculature hypotonic and large bilateral inguinal herniae were present. No clinical rickets and no oedema. Teeth small with black edges. Faint systolic murmur over the apex. BP 80/50. Liver and spleen not enlarged. X-ray showed no rickets or osteoporosis. Bone marrow puncture showed cystine crystals (see Part 8). Slit-lamp examination revealed widespread crystalline deposits in cornea and conjunctiva.

Chemical findings and haematological data. Summarising the most important findings, the plasma urea varied considerably between normal and high levels, the phosphorus between low and normal values. Serum calcium was usually normal and occasionally low, and phosphatase slightly raised or normal. Hypopotassaemia of 2.7 and 2.8 mEq/l was present in the two specimens tested for potassium. There was sometimes severe acidosis, during which the child's ammonia production was increased, but he lost abnormal amounts of bicarbonate in the urine, which did not show a pH of less than 6.2. Aminoaciduria was moderate with an excretion of 10-15 aminoacids in the urine. The plasma levels of various aminoacids were shown to be raised. Glycosuria was intermittent and slight with at times some ketonuria. Glucose was identified by chromatography. Occasionally pus cells and traces of albumen were found in the urine. The urine volume was increased up to 2 litres and the specific gravity ranged from 1001-1004. A test meal showed a low acidity of the gastric juice, with absence of free acids until after the administration of histamine. On a mixed diet the faecal fat content of two single specimens was 40.7% and 20.3% of the dried faeces. Pitressin had no effect on the daily urine volume (1500-2000 ml.). Thymol turbidity 1.5 units. No abnormal urobilinogenuria. There was at times a normochromic anaemia with 8.8 g.% Hb and 3.4 mill. red cells. Leucocytes and differential counts were normal.

Course of the disease and treatment. He received 100 ml. daily in 5 separate doses of the following solution : citric acid 140 g., sodium citrate 100 g., distilled water to 1 litre. In addition he had calciferol, 30,000 units daily by mouth ; ferrous sulphate and dilute hydrochloric acid shortly before meals ; blood transfusions and heparglandol at intervals. Under this treatment the general condition and appetite improved considerably. The CO_2-combining power and urea both returned to normal levels. Aminoaciduria and glycosuria ceased, but polydipsia and polyuria remained unchanged. After six months he had gained 4 lbs. and grown 2¼ inches. Vomiting and constipation had ceased completely and the photophobia appeared to be less troublesome. Perhaps the most striking change was that he could now walk half a mile or more whereas previously he was scarcely able to walk at all. A biochemical check-up of the plasma showed the CO_2-combining power to be 29.1 mEg/l., phosphorus 2.6 mg.%, calcium 11.7 mg.%, phosphatase 13.86 units, urea 28.6 mg.% and potassium (flame photometer by Dr. Barclay) 2.75 mEq/l. In view of the slightly increased calcium level in the plasma, calciferol was reduced to 10,000 units daily though there was no calciuria or other signs of vitamin D intoxication.

ABSTRACT OF THE CLINICAL FINDINGS IN CASE 6

D.S., male, age on first admission 15 mths. Lignac-Fanconi disease, subacute form, with rickets and variable biochemical findings.

We are indebted to Dr. Thursby-Pelham, North Staffordshire Royal Infirmary, Stoke-on-Trent, for permission to publish a short summary of his case record. A detailed report by Dr. Thursby-Pelham will be published at a later date.

Family and Case History. Healthy family ; no consanguinity. The urine of 9 relatives was tested for aminoaciduria and glycosuria by paper chromatography with negative results. The boy was healthy up to the age of 10 months, when he began to vomit, to lose weight, to refuse all solid food and to suffer from continuous thirst. Glycosuria was found, and he was referred to the North Staffordshire Royal Infirmary as a case of "diabetes" and admitted on several occasions during the last three years.

Admission Findings. Weight at 15 months 18 lbs. 7 oz., at 3 years 23 lbs. 2 oz. Small, pale and dehydrated, with active rickets and muscular hypotonia. Liver and spleen not enlarged. After cystine crystals had been isolated in the urine by Dr. McCall the diagnosis "Fanconi Syndrome" was established.

Chemical and other Investigations. An excess of 10-15 aminoacids in the urine was demonstrated by means of paper chromatography by Dr. Dent, University College Hospital, London, and a year later these findings were confirmed by one of us on several more urine specimens. The same technique showed the plasma aminoacid level to be raised. The reducing substance in the urine was identified as glucose. A sugar tolerance test proved the glycosuria to be renal. Cystine crystals were found in the bone-marrow and, by slit-lamp investigation, in the cornea and conjunctiva. The blood chemistry showed urea values to be increased or normal and phosphorus normal or low, whereas the phosphatase was always high and the calcium normal. There was no phosphaturia or calciuria in 24-hour specimens. The acidosis varied in severity and the child was able to excrete an acid urine (pH 5.0) with an increased ammonia coefficient. A pituitrin test had no effect on the polyuria.

Course of the disease and treatment. In the two years following the first admission no great change occurred in the child's chief symptoms. Ten months ago active treatment was started with high doses of vitamin D and sodium citrate-citric acid mixture. Since then he has definitely improved, has gained 4 lbs., and has grown 1 inch, his height being now 2 ft. 10½ ins. at 3½ years. The aminoaciduria and glycosuria have ceased. The rickets has healed, the child is lively and cheerful and is running about.

CLINICAL RECORD OF CASE 7

O.R., male, born 10.4.48, age on admission 2 yrs. 3 mths. Lignac-Fanconi disease in an early stage, with active resistant rickets and craniostenosis of the scaphocephalic type. Constitutional disturbance mild. Death during cranioplasty.

Family History. No consanguinity of parents. The patient's mother had two miscarriages. Three siblings are alive and appear well. Other members of the family are healthy, but none of the patient's relatives were tested for aminoaciduria or glycosuria.

Case History. Except that the shape of his head was abnormal, the child seemed quite healthy up to the age of 15 months, when he began to walk and it was observed that his legs were bowed. He had a teaspoonful of cod liver oil daily

during the last 4 months before admission ; none previously. No history of exceptional thirst or vomiting, but growth was retarded. Appetite remained good until about 3 weeks before admission, when it failed. He was admitted to the Bristol Royal Hospital for Sick Children for investigation under the care of Professor A. V. Neale, by whose kind permission we publish the case record.

Admission Findings. Weight at 2 years 3 months, 23 lbs. 1½ oz., height 32 inches. Apart from dwarfing his appearance was not that of an ill child. Intelligence normal. Hair fairly thick and brown. Head scaphocephalic (circumference 50 cm.) with a prominent occiput and bilateral proptosis (Fig. 3). Both legs showed lateral curving and there was some thickening of the epiphyses at the wrists. Cranial nerves normal apart from the optic nerve (see later). Both legs slightly spastic with increased reflexes and bilateral Babinski responses, but this did not appear to interfere with walking. Liver and spleen not enlarged. X-ray showed broadening and cupping of the metaphyses of most of the long bones and a coarse bony texture. Skull elongated in its antero-posterior diameter, increased convolutional markings. The sutures were united and the sella turcica appeared large. The skull changes were those of craniostenosis with considerable increase in intracranial pressure (see Part 6). Bone marrow puncture showed a few but typical cystine crystals (see Part 8). Ophthalmological findings : both optic discs showed early atrophy with slight but definite swelling. With some difficulty a slit-lamp examination was carried out, but no crystals or other abnormalities were found in cornea, iris or aqueous humour.

Chemical findings. To summarise the most important laboratory findings, the serum phosphorus was always low, the calcium normal, the alkaline phosphatase high. A 24-hours urine specimen demonstrated normal phosphorus and low calcium excretion. The plasma bicarbonate level revealed only a moderate acidosis, whereas the pH of the urine was 7.3 and the ammonia excretion normal. In the urine the aminoacid excretion was moderately increased, as shown by paper chromatography and by a raised aminoacid coefficient. Traces of reducing substances in the urine were detected occasionally and were identified by paper chromatography as glucose. Slight ketonuria, albuminuria, and the presence of pus cells in the centrifuged deposit were noted intermittently. The urine volume appeared normal and the fluid intake was rarely more than 900 ml. The specific gravity varied between 1004 and 1024. The chemical investigation was not completed because of the child's unexpected death. C.S.F. : pressure over 300 mm. H_2O, normal content of protein, sugar, calcium (5.2 mg.%) and phosphate (1.2 mg.%). Gastric acidity : free HCl and total acids present in normal amounts.

Course of disease and treatment. During his stay in hospital the child remained active and happy. Several short febrile attacks occurred with readings up to 102° F. but without obvious explanation. He was given increasingly large doses of calciferol and when on a daily dose of 30,000 units showed some healing of the rickets. The phosphatase level in the plasma fell whereas the phosphorus level remained low. No conclusion was reached as to whether the skull deformity was due to a platybasia secondary to rickets or to an independent abnormality (craniostenosis). Whatever the cause, it was considered necessary to relieve the high intracranial pressure. The child died during the operation of cranioplasty.

Autopsy findings. The cerebral convolutions were somewhat flattened, with coning of the cerebellar tonsils within the foramen magnum. The skull sutures were almost fully united and the calvarium markedly ridged. The other abnormal naked eye findings were well-marked antero-posterior bowing of the femur and tibia

(convex forward) with posterior buttressing. Apparently normal osteochondral junctions. Pallor of most organs. Otherwise the organs, especially liver and kidneys, appeared normal to the naked eye.

Histological examination showed swelling of the Kupffer cells, which had a somewhat foamy cytoplasm, a moderate reticulo-endothelial hyperplasia in the spleen and very slight changes in the kidneys, i.e. oedema of the interstitial tissue, degenerative changes in the tubular epithelium and slight thickening of the basement membrane of the glomeruli (for details see Part 8).

CLINICAL RECORD OF CASE 8

M.R., female, born 22.12.43, age on admission 6 yrs. 6 mths. Lignac-Fanconi disease with slight rickets. Chronic form, constitutional disturbance mild.

Family History. No consanguinity of parents. Two siblings died apparently of the same disease (photophobia, dwarfism, vomiting, thirst, etc.), one at 2½ years, the other in a tetanic crisis at 7 years. The patient's mother had renal glycosuria during diphtheria and two of her relatives suffered from diabetes mellitus. Fourteen members of the family were tested for aminociduria and glycosuria by paper chromatography with negative result.

Case History. For the first 6 months the child appeared healthy. She sat up at 5 months and walked at 10 months of age. At 6 months she had gastro-enteritis, and never regained health. Her appetite became fickle, severe attacks of vomiting occurred at intervals of one or two weeks and she ceased to gain weight and to grow properly. From the age of two she spent half her time in hospital. Photophobia developed at 3½ years, and she suffered from frequent headaches. She drank about 3 pints water daily, but her thirst was never as pronounced as that of the dead siblings ; it was said to have decreased during an attack of measles. According to her mother she often appeared hot. Nevertheless she was active and intelligent. There was no tetany, fracture or rickets. She received between 1 and 4 drops of cod liver oil daily until 8 months old, and during her fifth year 3 teaspoonfuls weekly.

Clinical and other findings prior to admission. (We are indebted to Dr. Everley-Jones for the following data and for transferring the child to our care). At 15 months, blood urea 48 mg.%, phosphorus 3.75 mg.% creatinine 1.28 mg.%. At 17 months, mild albuminuria in numerous urine tests, but no sugar. Fluid intake 30-40 ozs. daily (850-1100 cc.). At 3 yrs. 3 mths. bilateral otitis media. Weight 21 lbs. 3 ozs., height 32½ ins. BP. 90/60 mm.Hg. Blood urea 60 mg.%, phosphorus 2.2—2.5 mg.%, calcium 11.2—12.1 mg.%. X-ray showed slight osteoporosis. Retrograde pyelogram showed normal kidney pelves. Fluid intake up to 90 ozs. daily. At 3 yrs. 9 mths. there was less thirst and polyuria. BP.98/46. Blood urea 35 mg.%, calcium 9.7 mg.%, phosphorus 2.7 mg.%. At 4 yrs. blood urea 43 mg.% At 5 yrs. blood urea 47 mg.%, calcium 10.1 mg.% and phosphorus 2.6 mg.%. Alkaline phosphatase 16 units. At 6¼ yrs. blood urea 46 mg.%, calcium 9.5 mg.%, alkali reserve 21.6 mEq/l. When chromatography showed aminoaciduria, the child was admitted to the Children's Hospital, Birmingham, on April 24th, 1950.

Admission findings. At 6 yrs. 6 mths. weight 24 lbs., height 36½ ins. Active but delicate, pale, fair haired child, with marked photophobia (see Fig. 2). Dwarfing proportionate without bone deformities or clinical evidence of rickets. No oedema. Liver and spleen both palpable one finger-breadth below the costal margin. BP.90/65. X-ray showed mild rickets with little decrease in bone density. No bony deformities or fractures. Slit-lamp examination showed heavy crystalline deposits

widely distributed in the cornea and conjunctiva. Biopsy of conjunctiva showed small doubly refractile prisms, which were identified by X-ray crystallography as cystine (see Part 6). Bone marrow puncture also showed numerous cystine crystals (see Part 8).

Chemical findings. To summarise the most important findings, the plasma urea values were either increased or normal and the phosphorus levels were sometimes high and sometimes low. Calcium and alkaline phosphatase were normal. Rickets was shown to be due to a pathological loss of calcium and phosphorus in the faeces. Acidosis was only mild, the ammonia formation in the kidney normal or slightly raised and the urine had a pH of 6.1 or more. In the urine the excretion of 10-15 aminoacids was moderately increased, as was the plasma level for various aminoacids. Hypopotassaemia of 3.1 mEq/l or less was present in 7 specimens tested. Urine volume was increased up to 2200 cc. and the specific gravity ranged from 1005-1010. Pus cells, red blood cells, traces of albumen and traces of sugar were found occasionally in the urine. The sugar was identified by paper chromatography as glucose. Tests of liver function : Thymol turbidity 1.5 units, total cholesterol 233 mg.%, free cholesterol 56 mg.%, fibrinogen 0.40—0.44 g.%. No urobilinogenuria. Kidney function : Urea standard clearance averaged 43.1% of normal. A water concentration test with a reduced water intake of 390 ml. over 24 hrs. still gave an output of 1309 ml. The highest specific gravity observed in this test was 1010. Gastric acidity : First test showed no free HCl, whereas in the second test free HCl was present but only in small amounts. Fat balance : First test showed that 97% and second test that 89% of the fat intake was absorbed. E.C.G. : Long QT-interval (.450 sec.), S-T_2 depressed and T flat-topped. Suggestive of hypopotassaemia.

Course of disease and treatment. During the first weeks in hospital she vomited frequently, ate little but drank enormously. Otherwise she was active and companionable and soon became a general favourite. Alkali therapy with sodium citrate 100 grams, citric acid 140 grams, and distilled water to 1 litre was given in daily doses of 5×8 ml. up to 4×20ml. On this treatment the CO_2-combining power of the blood became normal, the aminoaciduria and glycosuria gradually disappeared and at the time of writing have not been evident for months. Her appetite improved and vomiting became rare. The rickets was cured with 50,000 units calciferol daily, followed by a maintenance dose of 10-30,000 units daily by mouth. To provide an adequate mineral intake, 1 gram calcium phosphate was given daily. Phosphorus and phosphatase values in the plasma became normal. After several low plasma potassium findings, potassium chloride (2g. daily) was given with a further improvement in general condition and complete cessation of vomiting, though thirst has remained unchanged. Mild anaemia was readily cured with iron given orally. Gastric anacidity was treated with drops of dilute hydrochloric acid before meals. After a year's stay in hospital the child's general condition has considerably improved, especially her appetite. She has gained 4 lbs. and grown 1¼ ins. She has been discharged home on the same treatment, though potassium citrate has been substituted for potassium chloride, which was refused because of its unpleasant taste.

CLINICAL RECORD OF CASE 9

M.B., female, born 1.7.40, age on admission 9 yrs. 9 mths. Lignac-Fanconi disease with rickets and severe osteoporosis, hypocalcaemia and tetany. Chronic type, death from Sonne dysentery, complicated by leptomeningitis, necrosis and haemorrhage of both suprarenals.

Family history. No consanguinity of parents. Two siblings died of meningitis and diphtheria, one other was stillborn. A paternal aunt died of an unknown disease, a maternal aunt of influenza at 8 years. Thirty-three members of the family were tested for animoaciduria and glycosuria with negative result.

Case history. For the first 18 months development was normal. She then became less lively and gained little weight. At 2 years she had her first attack of vomiting, which recurred frequently until her 5th year. Growth was delayed after 2 years and ceased at 4 years, when knock-knees was first noticed. Later she developed photophobia, and a number of pathological fractures occurred. Longstanding orthopaedic treatment for the limb deformities was unsuccessful. During the last 2 years of life she had an increasing number of tetanic attacks and developed thirst and anorexia. Her bowels were normal, and no fever was noticed.

We are indebted to Dr. Parry Williams for the data relating to this child before her admission to the Birmingham Children's Hospital and for transferring the child to our care.

Chemical findings prior to admission. Blood chemistry at 7 yrs. 9 mths. : urea 114 mg.%, calcium 7.8 mg.%, alkaline phosphatase 36.8 units, urea standard clearance 38% normal. Dye excretion : neither kidney excreted or concentrated dye. Urine : albumen, sugar, acetone were present, specific gravity was never over 1010. Blood chemistry at 8 yrs.: urea 110 mg.%, calcium 5.0 mg.%, phosphorus 4.4. mg.%. Blood chemistry at 8 yrs. 6 mths. : urea 69 mg.%, calcium 7.5, phosphorus 5.3 mg.%, albumin 5.5. and globulin 3.4 g.%. Urine : acid, traces of albumen and sugar, a few red blood cells and pus cells, scanty granular and hyaline casts. Culture sterile. Faeces : total fat 8.7% of dried faeces. At 9 yrs. 9 mths. alkali reserve in the blood 17 mEq/l. Urine sent to us for chromatographic investigation revealed a pathological aminoaciduria. On February 28th, 1950 the child was admitted to the Children's Hospital, Birmingham.

Admission findings. At 9 yrs. 9 mths. weight 30 lbs. 12 ozs., height 38 ins. Dwarfed, pale-skinned girl with fine, scanty hair who looked about four years old. Marked photophobia, cornea on both sides appeared to be slightly opaque. Teeth discoloured and the enamel defective. A "saddle" nose and knock-knees were the only bony deformities. She had difficulty in standing and walking and her muscles were markedly hypotonic. Liver and spleen just palpable. No oedema nor was the skin turgor reduced. Nails not remarkable. B.P. 110/75. X-ray showed moderate rickets but marked decrease in bone density with scanty, widely spaced trabeculae, especially at the lower ends of the femora. There were deformities (curvature of the tibiae). Looser zones and fractures. Slit-lamp examination showed heavy widespread deposition of crystals in cornea and conjunctiva (see Part 6). Bone marrow puncture showed numerous cystine crystals (see Part 8).

Chemical findings and haematological data. To summarise the most important findings, the urea in the plasma was always increased, though rarely over 200 mg.%. There was hypercholesteraemia. Phosphorus levels varied from high to low values, whereas the calcium levels were persistently low (under 7 mg.%), except that they rose under intravenous calcium therapy, while at the same time the phosphorus value dropped sharply to 1 mg.% or less. Balance studies showed that there was no increased phosphaturia or calciuria though the kidneys were able to excrete phosphorus in normal amounts. Serum phosphatase was slightly raised. Acidosis was constant but moderate, the ammonia formation in the kidney was decreased and the urine had a pH of 6.2 or more. Paper chromatography revealed

an increased excretion in the urine of 15 or more aminoacids, and the plasma levels also seemed to be raised. A reducing substance was frequently found in the urine and chromatography showed it to be glucose. Urine volume normal, specific gravity 1005-1011. Acetone, albumen, pus cells, red blood cells and hyaline casts were occasionally found. Normochromic anaemia, reticulocyte count less than 0.1 % toward the end. At that time the bleeding time was 4 min., the clotting time 4½ min. and she suffered from haemorrhagic diathesis. The clot retraction was poor and the prothrombin time 29 sec. (control 20 seconds—modified MacPherson technique). Test meal showed histamine refractory anacidity with little total acids in the juice. Liver function test showed thymol turbidity 4 units, bilirubin 0.8 mg.%, albumin 4.6 g.%, globulin 3.7 g.%. Urobilinogenuria in several specimens increased. Total plasma cholesterol 482 mg.%, free cholesterol 66 mg.%, ester being 86%. Faecal fat : the total fat content of a single dried stool specimen was 33.5%. E.C.G. : P-R O. 14 sec., T flat in lead III, but normal in I and II.

Course of the disease and treatment. At the end of the second month of her stay in hospital the patient contracted Sonne dysentery. The clinical picture of a Waterhouse Friderichsen syndrome developed with fever up to 103°F, leucocytosis of 42,500, haemorrhages into skin and from the nose, coma, a sudden drop of the plasma sodium chloride to 364 mg.%, but unaltered excretion of chlorides in the urine. The situation was further complicated by acidosis with a CO_2-combining power of 9 mEq/l. and tetanic crises associated with a hypocalcaemia of 6.8 mg.%. Intravenous treatment with calcium, alkali, suprarenal hormones, blood and plasma transfusions, salt infusions, etc., achieved a temporary improvement, but death finally ensued.

Autopsy findings. Cystine deposits could be seen with the naked eye as a fine white deposit in various organs, especially in the spleen. The presence of pathological amounts of cystine in this organ was proved by chromatography of an extract of the organ and by X-ray crystallography (Part 8). Haemorrhagic diathesis was widespread. The bones showed pronounced rachitic changes. The kidneys were severely contracted with uniform finely granular surface. The colon showed numerous ulcers throughout the whole length. Both suprarenals showed extensive necrosis associated with haemorrhages. An early purulent leptomeningitis due to Escherichia coli was also found. For detailed description and histological findings see Part 8.

ABSTRACT OF THE CLINICAL FINDINGS IN CASE 12

A.M., male, age on admission 7yrs. 3 mths. Lignac-Fanconi disease with rickets and severe dwarfism. Chronic form. Death following tracheobronchitis.

We are indebted to Prof. Linneweh, Marburg, for permission to publish this summary of his case, which has been published in detail elsewhere (LINNEWEH 1952).

Family and Case History. Two elder sibs died with cystine storage disease (PACHE 1940). Two other sibs are healthy and show no aminoaciduria. The parents are cousins. The child was normally developed at birth, but in early infancy he began to pass large quantities of pale urine, which was examined for cystine crystals with negative results. At the age of 12 months albuminuria was detected and this was confirmed at intervals up to his admission to hospital. Photophobia was present from the fourth year onwards.

Admission findings. At the age of 7 yrs. 3 mths. height and weight were those of a child of two, though his mental development appeared normal. Bone ends en-

larged as in rickets. Heart, lungs and abdominal viscera normal clinically. Optic fundi normal. Slit-lamp examination revealed depositions of crystals in cornea and conjunctiva. Bone marrow puncture showed numerous doubly refractile cystine crystals. No anaemia. Blood chemistry showed normal N.P.N. (24 mg.%), increased alkaline phosphatase (62 units), normal phosphorus (3.5 mg.%) and low calcium (7 mg.%). The urine contained traces of albumen and a few red blood cells, but no cystine crystals. There was marked polyuria with an average output of 5 litres in 12 hours of day. The specific gravity of the urine during a concentration test did not exceed 1008, whereas the fluid excretion in a water test was normal. Chromatographic investigations of a urine specimen from this child sent to the Children's Hospital, Birmingham, showed a strong general aminoaciduria with increased excretion of valine, the leucines, phenylalanine, tyrosine, proline, beta-alanine, aspartic and glutamic acid, alpha-alanine, threonine and glycine. The reducing substance in the urine was shown by paper chromatography to be glucose.

Course of the disease and postmortem findings. After 3 weeks in hospital the child contracted tracheobronchitis, to which he succumbed. At necropsy cystine storage was found in liver, spleen, kidneys, lymph glands and bone marrow. There was fibrosis and marked atrophy of both kidneys. Other findings were tracheobronchitis, moderate dilation of the right ventricle of the heart, partial atelectasis and oedema of the lungs, larynx and brain.

ABSTRACT OF THE CLINICAL FINDINGS IN CASE 13

M.L., female, age on admission 13 mths. Lignac-Fanconi disease. Rickets with low serum calcium and normal phosphorus. Subacute form seen in an early stage.

We are indebted to Dr. Boehncke, Hamburg, for permission to publish a summary of his case, which is being prepared for detailed publication in the *Zeitschrift für Kinderheilkunde.*

Family and Case History. The parents are first cousins. One miscarriage preceded the birth of this child. Development proceeded normally until the 10th month. She received generous amounts of vitamin D and also ultraviolet radiation. She then began to vomit, to refuse food and develop increasing thirst.

Admission findings. At 13 months she was 3½ lbs. underweight with attacks of fever. She had fair hair but no obvious photophobia. Fontanelle was closed and there was no clinical rickets but rachitic bone changes were demonstrated by X-ray investigation. B.P. normal. Slit-lamp investigation revealed numerous crystalline deposits in cornea and conjunctiva and biopsy of the conjunctiva showed crystals characteristic of cystine. Bone marrow puncture also showed numerous doubly refractile and in part hexagonal crystals. Investigation of the blood chemistry showed a normal phosphorus, normal or moderately raised N.P.N. and low calcium (8.1 mg.%), with occasional attacks of tetany. Urine : specific gravity 1008 to 1016, reaction nearly always acid, albuminuria, with red blood cells, pus cells, and granular casts in the centrifuged deposit. Ammonia production increased. Occasional glycosuria of renal origin. Flat blood sugar tolerance curve. Specimens of urine and plasma which were sent to us for chromatographic investigation showed in the urine a general aminoaciduria with as much as a twentyfold increase of valine, the leucines, tyrosine, phenylalanine, proline, lysine and other aminoacids. Similar chromatographic findings were obtained by Dr. Souchon, Kiel. The aminoacid

pattern of the plasma was the same as in normal plasma, but quantitative microbiological studies by Professor Krebs and Dr. Schreier showed plasma levels for glutamine, glutamic acid and other aminoacids to be raised or on the upper limit of normal. There was a considerable increase of the total cystine content in the serum as estimated by the method of SULLIVAN and HESS using Folin's reagent. The total cystine content was also increased in the urine. The free cystine content of the serum and urine estimated by microbiological assay by Schreier was normal. Electrophoresis showed decrease in serum albumin and increase of the alpha and beta and to a lesser degree of the gammaglobulins. Electro-cardiography showed a prolonged Q-T interval. Retrograde pyelography was normal.

Course of the disease and treatment. This case has been under investigation for only a short time. The urine became alkaline and the rickets healed without administration of vitamin D or citrate or any other form of therapy. Later, when citrate therapy was started, the child improved clinically and aminoaciduria was reduced considerably. Unfortunately this therapy led to tetanic attacks and could not be carried out consistently.

ABSTRACT OF THE CLINICAL FINDINGS IN CASE 14

J.S., male, age on admission 2 yrs. 6 mths. Lignac-Fanconi disease with rickets and other typical findings, but with an acid urine. Subacute form.

We are indebted to Dr. C. Harrison Snyder, New Orleans, for permission to publish this summary of his case.

Case History. The boy was healthy up to the age of 12 months, when he had a short attack of diarrhoea. At 14 months he ceased to gain weight, developed polydipsia and polyuria, and showed rickets with bowed legs, despite high doses of vitamin D.

Admission findings. At the age of 2 yrs. 6 mths. the boy's size and weight were those of a child of 12 months. He had fair hair and showed photophobia. Clinical and X-ray examination showed well-developed rickets with bowing of both tibiae, beading of the costochondral junctions and swelling of the end of long bones. Bone marrow puncture showed numerous crystals characteristic of cystine. The blood chemistry showed a low phosphorus, normal calcium and urea, and a slightly raised alkaline phosphatase. Fasting blood sugar normal. Marked acidosis of 12.2 mEq/l. CO_2-combining power in the serum, and the pH of the urine was never above 6.0. Daily output of urine between 2 and 3 litres, not decreased by pitressin. The specific gravity did not exceed 1003. The urine consistently showed albumen and occasionally traces of reducing substances, which were identified by chromatography as glucose. Examination of the urinary sediment essentially negative. Total excretion of phenol-sulfonephthalein in two hours 30%. BP normal. The urine specimens sent to us for chromatographic investigation showed a gross general aminoaciduria, with an increase in valine, the leucines, lysine, serine, tyrosine, threonine, proline, phenylalanine, alanine, glutamine and glycine.

Course of the disease and treatment. The child has been treated with Vitamin D, supplementary calcium, sodium citrate and Vitamin B-complex. The rickets has healed almost completely. The polyuria and polydipsia are somewhat relieved. Blood chemistry, albuminuria and hyposthenuria are unchanged. Growth remains stunted, but weight has increased by 2 lbs.

PART 4 : THE RADIOLOGY OF LIGNAC-FANCONI DISEASE

by

R. ASTLEY, C. G. TEALL and **H. BICKEL**

from

THE CHILDREN'S HOSPITAL, BIRMINGHAM, AND THE DEPARTMENT OF PAEDIATRICS AND CHILD HEALTH, UNIVERSITY OF BIRMINGHAM

The seven children with Lignac-Fanconi disease that are considered in this paper showed radiological appearances which may be summarised in three groups :

1. Normal bone structure ;
2. Simple rickets ;
3. Predominant osteoporosis.

Group 1. Normal bone structure

Two children (Case 4, age 15 months ; Case 5, age 2 years) showed neither rickets, nor lack of bone calcium. Bone structure and form were entirely normal. Carpal ossification was considerably retarded in Case 4 and in Case 5 it was at the lower limit of the normal range for his age (VOGT and VICKERS 1938).

Intravenous urography indicated that Case 5 had impoverished renal function but no macroscopic structural abnormality. In Case 4, rickets was said to have been shown by X-ray elsewhere at the age of six months. Case 5 had also been examined elsewhere a year previously, but with normal findings.

Group 2. Simple rickets

Four children presented in this group, indistinguishable from primary avitaminosis—D :

Case 1, age 14 months. Marked rickets with considerable "cupping" ; only slight hypocalcification ; normal carpal ossification.

Case 2, age 17 months. Moderate rickets with some "cupping" ; marked skeletal hypocalcification ; normal carpal ossification.

Case 7, age 2½ years. Slight rickets with a little "cupping" ; changes more evident at the knees than at the wrists. Only mild hypocalcification. Carpal ossification within normal limits. Scaphocephalic craniostenosis.

Case 8, age 5½ years. Mild rickets without "cupping" ; mild skeletal hypocalcification ; white lines of disturbed bone growth ; retarded carpal ossification.

No child in this group showed fractures or pseudo-fractures, nor were there deformities of softened bone. The trabecular pattern was close-set or only slightly coarse. Hypocalcification was manifest by a lack of radiographic contrast between cortex and spongiosa, the appearance that is typical of the osteoid-coated bony framework in simple rickets. Skull pictures were normal, except in Case 7 (Fig. 1) where there was a craniostenosis.

In three children of this group (Cases 1, 2 and 8), healing of the rickets occurred with heavy dosage of Vitamin D, leaving a mild deficiency of skeletal calcium and a zone of density at the bone ends that consisted of fine, close-set trabeculae. In

Case 2 the process of healing appeared to be intermittent ; two parallel lines of density were deposited in the osteoid zones of the bone ends and then the intervening less dense areas were filled in by a dense bone of very close pattern (Fig. 2).

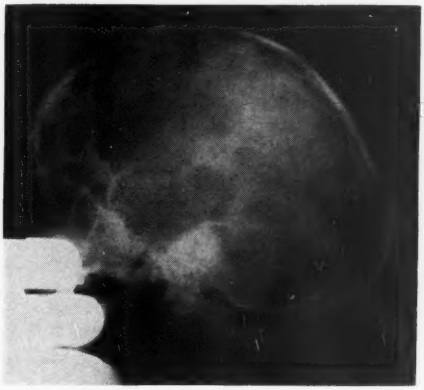


Fig. 1. Case 7, age 2¼ years. Craniostenosis.

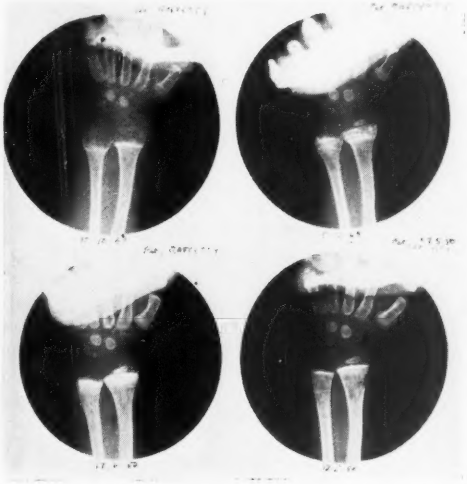


Fig. 2. Case 2, age 17 months. Group 2 : simple rickets.
Note the healing under heavy dosage of Vitamin D.

The alimentary tracts of two children (Cases 1 and 8) appeared normal when examined by a non-flocculating mucus-resistant barium sulphate suspension (ARDRAN et al 1950 ; ASTLEY and FRENCH 1951).

Group 3. Predominant osteoporosis

One child (Case 9, age 9 years) showed marked lack of skeletal calcium, deformities, fractures and pseudo-fractures. The fractures, which occurred for trivial reasons, united well and in normal times. The pseudo-fractures showed themselves especially along the concave borders of the bowed leg bones. The bone pattern consisted of scanty, widely separated trabeculae in the lower ends of the femoral shafts, where the cortex was poorly defined and the background density of the bone little greater than that of the soft tissues (Fig. 3). Similar changes, to a less marked degree, were seen elsewhere, particularly in the proximal tibiae (Fig. 4). There was no club-like widening of the bone ends of the type described by FANCONI (1946) and skull X-rays were normal.

Associated with this gross deficiency of calcium was a moderate degree of rickets with "cupping." Carpal ossification was much retarded.

Discussion

In descriptions of bone disease considerable confusion is apt to arise from the indiscriminate use of the word "osteoporosis." According to BAKER (1939) it indicates rarefaction of bone brought about by an excess of osteoclastic over osteoblastic activity. The total bony

Figs. 3 and 4. Case 9, age 9 years. Group 3 : predominant osteoporosis ; moderate rickets. The trabeculae are scanty but sharply defined. Healed pathological fracture of right femur. Curvature of tibiae and fibulae.

tissue is reduced in amount but what remains has a normal calcium content. In contrast, the term "hypocalcification" indicates incomplete calcification of bone matrix, i.e. the presence of osteoid tissue. A given fragment contains an abnormally low calcium content. Both osteoporosis and hypocalcification result in reduced X-ray density ; an attempt is made to distinguish between them in the following account.

Examination of the previously recorded instances of Lignac-Fanconi disease shows that probably all can be classed into one of the three groups in which the present cases have been described. There are very few examples, apart from those in the present series, that fall into the first group, with no bone changes, probably because the diagnosis has not been entertained in such circumstances. Those existing are not altogether satisfactory (e.g. RUSSELL and BARRIE 1936, Case 2 : treated for rickets from 18 months to 7 years of age, but bones not examined at autopsy ; RÖSSLE 1938 : no X-ray examination but costochondral junctions macroscopically normal at autopsy).

The majority of the children fall into Groups 2 and 3, with all gradations between them. Group 2 has the X-ray appearance of simple rickets, indistinguishable from the primary avitaminosis. There is a variable degree of cupping and widening of the bone ends ; the trabecular pattern is often close-set and hypocalcification is manifest by lack of radiographic contrast in bone structure ; in extreme cases fractures may occur.

In Group 3 there are the appearances of long standing late or "resistant" rickets. There is now true osteoporosis instead of hypocalcification ; lack of radiographic density is associated with a wide-spaced, scanty but clearly defined trabecular pattern, and with fractures, pseudo-fractures and softening deformities. The rickets at this stage may be relatively mild. Indeed, FANCONI (1946) and FANCONI and BICKEL (1949) have described three children with osteoporosis but without rickets. Fanconi referred to the changes as a typical " osteodysplasia " and believed that it was a specific bone lesion of Lignac-Fanconi disease, directly related to the inborn error of protein metabolism. His description appears to be principally that of considerable osteoporosis plus club-like enlargement of the bone ends, an appearance not mentioned by other writers, nor found in the present series.

By his kindness we have been able to study contact prints of these X-rays. While agreeing that they show no evidence of active rickets, in two instances we feel that the appearances suggest the probability of past rickets which has healed.

To explain the presence of rickets in some children but its absence in others, FANCONI (1946) postulated that the osteoporosis ("osteodysplasia") was the original lesion, with the later superimposition of avitaminic rickets and, later still, of renal rickets. Yet this cannot explain the existence of Group 2, with simple rickets but the absence of true osteoporosis. Rickets occurs when tertiary calcium phosphate is deposited so slowly that new bone production exceeds it in rapidity and consequently osteoid tissue is produced (HOLT et al. 1925). In Lignac-Fanconi disease such conditions are most likely to occur at the onset of the disease, before growth has ceased completely. Hence it seems more probable that changes similar to those of simple rickets are the original skeletal manifestation of the disease, with marked osteoporosis as a later superimposition, rather than in the reverse sequence. (In this connection the case of DEBRÉ et al. (1934) may be quoted ; at four years of age there was simple rickets but at eleven years there was also extreme osteoporosis. See also the addendum).

It is reasonable to argue that if the child lives long enough, the bone changes will tend to progress from those of Group 2 to those of Group 3. Growth is severely reduced or virtually ceases in such children and very little new bone matrix will be laid down. Hence osteoid tissue will be scanty and the X-ray signs of active rickets will become less marked (or even absent, as in Fanconi's cases). But osteoclastic resorption will continue, perhaps increased by secondary parathyroid hyperplasia, resulting in progressive osteoporosis. The trabeculae will now become scanty but well defined because they are no longer ensheathed in osteoid tissue ; the softening will lead to the appearance of deformities, fracture and pseudo-fractures.

The presence of predominant osteoporosis, indicating that there has probably been a long period of poor calcium deposition without appreciable growth, suggests a relatively less acute form of the disease. In support of this thesis, Group 3 cases described in the literature tend to be older than those of Group 2. (**Group 3** : FANCONI, 1946, age $4\frac{1}{4}$; DEBRÉ, 1934, age 11 ; GITTLEMAN, 1940, age 7 ; MCCUNE, 1943, age 10 ; JACOBSON, 1949, age 13 ; present series, age 9. **Group 2** : BEUMER, 1937, age 10 months ; PACHE, 1940, ages 2 and 5 ; DANIS, 1941, age 3 ; present series, ages 14 and 17 months, 2 years and $5\frac{1}{2}$ years.) In the acute, rapidly progressive type of the disease with gross metabolic disturbance before the occurrence of glomerular insufficiency, the stage of Group 3 may not be reached before death

intervenes (e.g. Case 2, with only moderate rickets, was acutely ill with severe acidosis and might easily have died).

In Part 7 the phosphorus and calcium balances of children with Lignac-Fanconi disease are analysed. Before treatment there is reduced intestinal absorption of these elements and especially of phosphorus. Contrary to previous conceptions, an increased loss of phosphorus and calcium through the kidney could not be found in any of the available balances, so that the theory of an impaired tubular reabsorption of these minerals is not substantiated by the evidence.

The bone changes are thus conceivably caused by a mechanism similar to that acting in other forms of resistant rickets, namely, defective intestinal absorption of phosphorus and calcium. Therefore the observation in Lignac-Fanconi disease of the radiographic picture of simple rickets, sometimes progressing to that of longstanding late rickets (but without any special distinguishing features) is in accord with theoretical considerations. When bone changes are absent, the defect in absorption is probably slight.

Differential Diagnosis

1. **Rickets and Resistant rickets.** As already stated, no radiological differentiation from these conditions is to be seen, nor is it to be expected. But avitaminic rickets is now a relatively rare disease ; therefore, when its X-ray appearances are presented the possibility of Lignac-Fanconi disease should be kept in mind.

2. **Renal rickets.** In the late stage of the chronic type of Lignac-Fanconi disease, when nitrogen retention occurs, the serum calcium

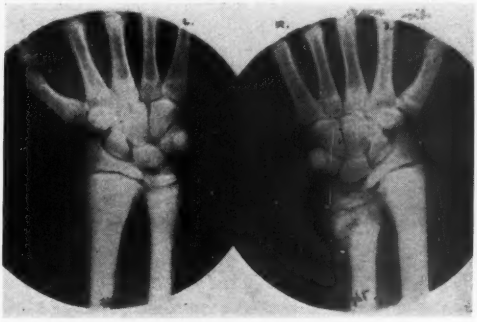


Fig. 5. The special type of radiographic appearances in renal rickets. The changes are asymmetrical (much more marked at the right wrist). They involve the whole metaphysis and not only the epiphyseal line.

begins to drop and the phosphorus to rise. Thus the biochemical state for a time approaches that of renal infantilism, differing in that the phosphorus, starting from a subnormal level, tends not to rise so high. It might be expected that the X-ray changes of renal rickets could occur in this terminal phase.

One of us (TEALL, 1928) described two types of rickets in renal dwarfism. In some there were bone changes which were indistinguishable from simple rickets. But in others the appearances were different (Fig. 5). The changes occurred throughout the metaphyses and rachitic fraying was not limited to the region of the epiphyseal line ; sometimes the end of the bone appeared like a wooden post that had rotted away in the earth. The distribution was not symmetrical ; one wrist might show gross abnormality and the other little or none, in contrast to the symmetrical involvement of the bones in other forms of rickets. This type of change we ourselves have not yet seen in Lignac-Fanconi disease, nor is it shown in the illustrations of cases in the literature, although by courtesy of Dr. BOEHNCKE we have seen a print from an X-ray film where the changes approached this type in the terminal phase, soon before death.

Summary

The radiological findings in seven children with Lignac-Fanconi disease are described and compared with those in the literature.

Bone changes may be absent. When present, they are those of simple rickets or, in the more chronic cases, of long-standing late rickets. The appearances are non-specific and not distinguishable from those of avitaminosis-D or from "resistant" rickets.

These observations agree with theoretical conceptions because the bone changes, contrary to previous belief, probably originate in a failure of intestinal absorption of phosphorus and calcium.

We wish to thank Prof. G. FANCONI and Dr. H. BOEHNCKE for sending us prints from the radiographs of their patients, and Prof. A. V. NEALE for permitting us to use the X-rays of Case 7.

Addendum

Since this paper was written we have seen the transition from simple rickets with hypocalcification to rickets with true osteoporosis that we postulated in chronic cases of Lignac-Fanconi disease. In a girl of 2 years the trabeculae were close-set but ill-defined due to osteoid ; but at 5 years they had become sharply defined and much further apart.

This child showed a feature not present in our other cases, namely, the trapping of non-calcified areas from the rachitic zones in the metaphyses during growth. At the wrists, knees and ankles the rachitic cupping was more pronounced at various points in the epiphyseal line, forming depressions which gradually became deeper. Eventually, in the course of 2 years, these depressions became separated from the epiphyseal line as rounded cyst-like translucent areas in the metaphyses (Fig. 6).

Fig. 6. Rickets with osteoporosis and genu valgum. There are translucent areas, some of them well defined and corticated, where non-calcified material from the rachitic zones has become trapped in the metaphyses during growth (see Addendum).

PART 5 : THE OPHTHALMOLOGY OF LIGNAC-FANCONI DISEASE

by

A. A. DOUGLAS and **H. BICKEL**

from

THE CHILDREN'S HOSPITAL, BIRMINGHAM, AND THE DEPARTMENT OF PAEDIATRICS AND CHILD HEALTH, UNIVERSITY OF BIRMINGHAM

Excellent and detailed accounts of the eye changes in this condition have already been given by BÜRKI (1941) and by MÜLLER in a paper by ULLRICH (1948), each of a single case. The ophthalmological appearances observed in the five cases (Cases 1, 2, 5, 8, 9 of this series) examined at the Children's Hospital, Birmingham, were in general very similar to those of BÜRKI and ULLRICH, except in one important respect (see under corneal epithelium). The following brief description of the findings in our patients is the first in the English language and special care has been taken over the illustrations. Moreover, a new method, X-ray diffraction photography, has been used to identify the crystals in a small conjunctiva biopsy.

Examination of the Eyes in Infants and young Children. In all our patients, especially in the early stages of the disease, the crystalline deposit in the eyes might easily have escaped detection if they had been investigated with the naked eye or with the loupe only. More detailed examination by the slit-lamp had to be carried out in every case. Such an examination presents considerable difficulties, as even docile and well-behaved infants and children under five are unable to keep their eyes sufficiently steady to permit of deliberate and detailed examination with the corneal microscope and slit-lamp, or even with the ophthalmoscope. The majority of normal infants struggle and cry, so that only a superficial examination is possible. In all cases, therefore, sedation was necessary, and 1—1.5 grammes of chloral hydrate was given rectally two hours before the proposed time of examination.

The hypnosis thus produced was always sufficient to permit examination and was often so deep that the child slept comfortably throughout with immobile eyes, thus permitting as deliberate and detailed an inspection as is possible in the case of a co-operative adult.

The child, suitably raised and supported by pillows, was held in position at the slit-lamp by a nurse, who herself sat in the chair usually occupied by the patient.

Ocular Appearances in Lignac-Fanconi disease

General appearance of the eyes

Conforming to the rest of the facial skin, the lids were pale or rather yellowish, the lid skin being delicate and a little more wrinkled than usual.

Some, but not all, of the patients had photophobia. Severe lacrimation was not a feature of any of them.

When the eyes were examined more closely in the well-developed cases, a person with good accommodation could just see that there was a very fine greyish stippling of the cornea (Fig. 1) and that there were white chalky-looking deposits in the bulbar conjunctiva near the corneal margin.

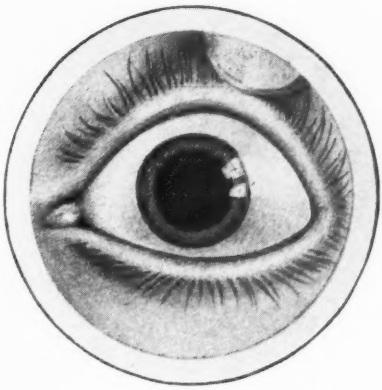


Fig. 1. Naked eye appearance of the crystalline deposits in the eyes of an advanced case of Lignac-Fanconi disease (Case 8).

Conjunctiva

With the loupe (x 10) the conjunctiva was seen to contain chalky-looking deposits. While these are present in all the visible parts of the membrane, they are most numerous in the fornices and near the limbus. When visualised with the slit-lamp and corneal miscroscope they appear granular, but here and there tiny points of brilliant coruscation, constantly changing in position as the eye or the beam moves slightly, indicate that crystals are present. Particularly at the limbus it can be seen that the deposits are specially related to the capillaries (Fig. 2). If the blood-vessels are compared to a branching bough, the deposits in their arrangement are not unlike masses of blossom growing from the twigs.

Even with the higher powers of the biomicroscope it was impossible to resolve these aggregations into their constituent elements. Small pieces of conjunctiva were therefore removed from the lower fornix and limbus of one of the patients (Case 8). These were examined

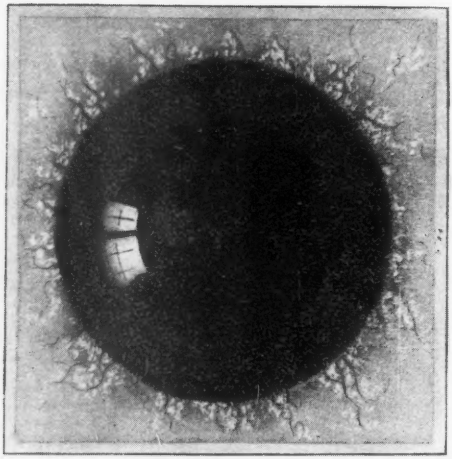


Fig. 2. Cornea and limbus as seen under low magnification. An area of epithelial dystrophy is seen between the centre and the window-reflections.

microscopically. We are indebted to Dr. BAAR, pathologist to the Children's Hospital, for the following report :

"This conjunctiva shows a normal stratified low columnar epithelium with a fair number of spherical goblet cells. The blood vessels of the lamina propria are distended and engorged. There is an increased cellularity of the superficial layer of lamina propria. These cells are lymphocytes, plasma cells and large mononuclear cells. Some of the latter have a spherical nucleus, others a kidney-shaped or an almost rod-shaped nucleus. Some show a foamy cytoplasm. Apart from the scattered cells there is also one lymph follicle with a germinal centre present. Scattered throughout the whole lamina propria are clusters of crystals. The number of crystals in one cluster varies between a few and about 50 or more. The crystals are either slender prisms or tiny plates. Some of the latter are clearly hexagonal though the edges are usually rounded. The crystals are birefringent. Many of these clusters of crystals, especially the larger ones, appear to be extracellular, but intracellular crystals are also seen and frequently crystals are seen surrounding one or two nuclei. I believe that all crystals were originally intracellular and that the extracellular clusters are the result of death of the cell within which they had been formed. No crystals were seen within the lymph follicle. The ninhydrin test gave a diffusely positive reaction." The biopsy findings are shown in Figs. 3 and 4.

In order to identify the crystals the method of X-ray diffraction photography was used. We are indebted to Dr. R. W. H. SMALL of the Department of Chemistry, University of Birmingham, for carrying out this method in two of our patients and for the following account :

Technique of X-ray Diffraction Photography

The identification of crystalline substance by means of its X-ray diffraction pattern is a relatively simple procedure. A small specimen of the material of dimensions 1 mm. $\times$ 0.5 mm. $\times$ 0.5 mm. is mounted on a special X-ray "camera." A fine beam of X-rays (preferably of single wave-length), obtained by passing the radiation through a series of pinholes, impinges upon the specimen. The X-rays which are diffracted by the crystal lattice are distributed uniformly over the surface of a series of cones with the crystal as apex and co-axial with the incident beam of X-rays. These diffracted X-rays may be recorded on a photographic film either placed perpendicular to the incident X-rays or more usually bent into the form of a cylinder whose axis passes through the specimen and is perpendicular to the incident X-ray beam.

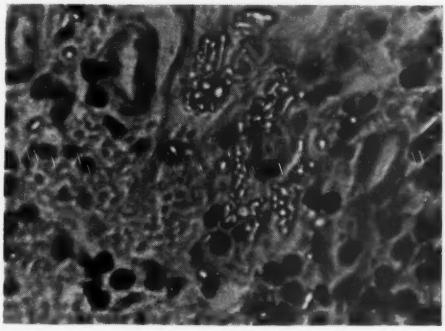


Fig. 3. Conjunctiva tissue of a biopsy specimen of Case 8.

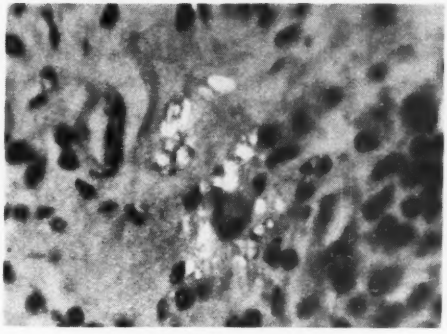


Fig. 4. The same specimen with crossed Nicols.

Exhibited Photographs

The X-ray photographs exhibited here have been recorded in this manner. It will be noted that the intersection of the cones of diffracted

X-rays with the film produces a pattern of rings. **This pattern is entirely characteristic of the crystalline structure** and only in the exceptional case of isomorphous substances would any difficulty be experienced in differentiation. It will be noted that cystine and methionine, although chemically somewhat similar, produce quite different X-ray patterns (Fig. 5). The identification of small quantities of crystalline cystine in the conjunctiva is obvious from the photographs (Fig. 6).

These photographs were taken on a cylinder of radius 3.90 cm. with copper K. radiation.

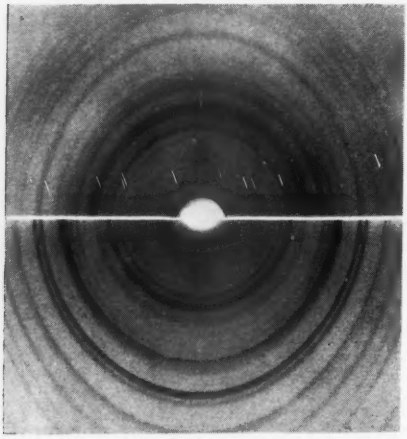


Fig. 5.

X-ray diffraction photographs showing pure crystalline aminoacids. Above is seen the diffraction of methionine ; below that of cystine.

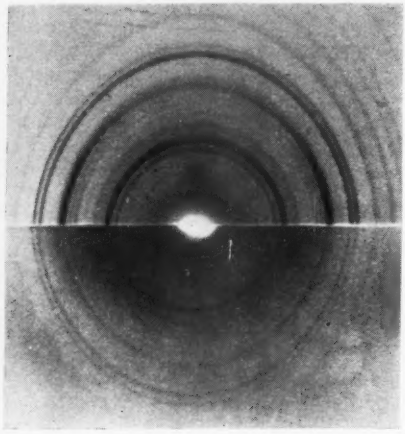


Fig. 6.

X-ray diffraction photographs showing pure cystine above, and below cystine in a biopsy specimen of conjunctiva from Case 8.

Cornea

It is this part of the eye which provides the most striking picture and that most characteristic of the disease.

Epithelium. In the previous descriptions the corneal epithelium is reported to be normal ; this was so in three of the five cases examined at the Children's Hospital, none of the three having severe photophobia. In the case of the other two, however (Cases 8 and 9), who both had constant and troublesome photophobia, there were well-marked abnormalities of the epithelium. These took the form of scanty greyish patches up to 2 mm. in diameter and staining deeply with fluorescin (Fig. 2). There was no ulceration or loss of epithelium, only what appeared to be a dystrophic change. This change, in our opinion, is the cause of the photophobia in these children.

Bowman's Membrane appeared to be normal but impinged upon by the crystals in the subjacent stroma.

Substantia Propria. When the cornea was examined with the loupe a fine stippling was observed. With the corneal microscope the appearances were striking, characteristic and beautiful (Fig. 2). The corneal stroma was packed with crystals which appeared white for the most part, but also showed a polychromatic lustre, the colours being subdued and delicate. Though microscopic examination of sections (see pathological section) shows the crystals to be actually rather short and thick, when seen by the biomicroscope in the living cornea they appear as short needles. So numerous are they that, when the cornea is examined as a whole by diffuse light, the picture is not unlike metal burnished by a coarse abrasive substance.

The optical section by the broad beam of the slit-lamp is shown in Fig. 7. In typical cases the distribution of the crystals at the periphery and at the centre is not the same. While at the periphery the whole thickness of the cornea is affected, in the centre the crystals occupy mainly the anterior third but are also present in scattered groups near the posterior surface. This is shown best by the narrow beam of the slit-lamp (Fig. 8). It should be noted, however, that in the case of one child aged 9 (Case 9) even the central parts of the cornea were packed with crystals from front to back.

Descemet's Membrane and the **endothelium** appeared normal in all the cases examined.

Iris. In two of the cases (Cases 8 and 9) the iris was seen to be the site of deposits of crystals. They were scattered over the anterior surface and produced an appearance suggesting that the iris had been decorated for a Christmas party with artificial frost.

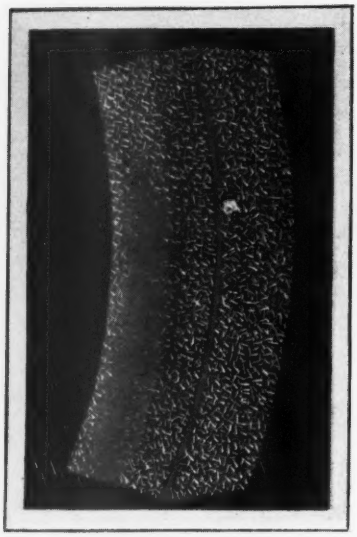


Fig. 7. Optical section through the cornea by the broad beam of the slit-lamp.

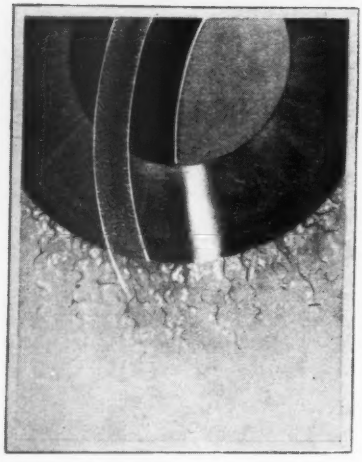


Fig. 8. Narrow beam of the slit-lamp showing the distribution of the crystals in the cornea.

The **lens** and **vitreous** were normal to examination with the slit-lamp. With the ophthalmoscope no abnormality was seen in any of the eyes examined. The crystals in the cornea were not visible with a +20 lens in the ophthalmoscope.

Differential Diagnosis

There is no corneal opacity occurring in childhood—or probably at any age—which need give rise to serious difficulty in diagnosis. The obvious crystalline nature of the deposits, their diffuse distribution in the cornea, their presence in the conjunctiva, and often also the iris, combine to produce a picture which is quite characteristic. Other causes of bilateral symmetrical corneal opacity in childhood are Hurler's syndrome and the familial corneal dystrophies. In Hurler's syndrome the lipoid deposits are amorphous. The granular type of familial dystrophy might conceivably give rise to confusion in an older child ; but in this condition the opacities, though sometimes suggesting a crystalline nature, are aggregated into crumb-like masses, occupy the central region of the cornea and not the periphery, and only the anterior third of its thickness.

We are greatly indebted to Mrs. MARY YOUNG for the skilful way in which she has drawn the figures, which, it must be understood, are semi-diagrammatic.

Summary

The ophthalmological appearances in five cases of Lignac-Fanconi disease have been studied. In every case crystalline deposits were seen in cornea and conjunctiva, in two cases in the iris, but never in the lens, vitreous or other media of the eye. Massive deposition was easily recognisable by loupe and may even be suspected by naked eye examination. In less developed cases the crystalline deposits were visible only by use of a slit-lamp ; the application of this technique in uncooperative children is described.

Microscopy of a conjunctiva biopsy specimen revealed that the crystals were partly hexagonal, their localisation being intra- and extra-cellular. The ninhydrin test gave a diffusely positive reaction. X-ray diffraction photography of the biopsy specimen showed the pattern to be entirely characteristic of pure crystalline cystine.

In two patients with severe photophobia epithelial lesions of the cornea were shown by staining with fluorescin. This change was absent in the patients without photophobia and is in our opinion the immediate cause of the photophobia.

The eye appearances in Lignac-Fanconi disease are sufficiently characteristic to eliminate any serious diagnostic difficulty.

PART 6 : DETAILED CLINICAL AND BIOCHEMICAL REPORT ON TWO BROTHERS SUFFERING FROM LIGNAC-FANCONI DISEASE

(Cases 10 and 11 in this series)

by

M. G. PHILPOTT, C. C. HARVEY and **E. FINCH**

from

THE DEPARTMENT OF CHILD HEALTH, UNIVERSITY OF SHEFFIELD, AND THE CHILDREN'S HOSPITAL, SHEFFIELD

The first mention of generalised cystinosis in the literature appears to have been made by ABDERHALDEN (1903), but it was not until the work of LIGNAC (1924 and 1926 a and b) that a syndrome of rickets with generalised cystinosis and glycosuria became recognised. SCHIER and STERN (1926) described rickets, glycosuria and hypophosphataemia occurring in the same patient, but without mention of cystinosis. More work has been done by FANCONI (1931, 1936, 1946, etc.) than by any other author to elucidate these conditions, and if personal names are to be given to the disease it is proposed that those of LIGNAC and FANCONI should be used.

We have recently had the opportunity of studying two brothers who presented all the important features of Lignac-Fanconi disease. Two siblings, both girls, had previously died and from their histories and other relevant data they too appear to have had the same disease. These cases have been investigated and appear to be worthy of publication, particularly in view of the unique family history, the genealogy it presents, the comparative longevity of Case 2, and the results of some special investigations.

Case Reports

The cases of two brothers are presented. Owing to physical stunting and curvature of the tibiae, Case 1 was referred to us from the school medical inspection. On inquiry it was learned that an elder brother was affected similarly and he constitutes Case 2 of this report. It was also learned that two sisters, who were severely affected by rickets, had died of pneumonia.

Family History : The parents are unrelated. The father is a bronchitic coal miner, aged 49, and the mother a healthy woman aged 46. Their parents had lived to advanced age. Case 1 is the ninth of ten children, and has healthy sisters aged 23 and 22 and brothers aged 16, 10 and 4. Another brother, aged 14, is Case 2, and the fourth and seventh children of the family, both girls, appear to have died of this same disease. The third child of the family (a boy) died at 3½ years of age of pneumonia, with no evidence of pre-existing disease.

In the diagram of the pedigree (Fig. 1) three generations are shown. Other than the four indicated, all members appear by inquiry to have been free from symptoms referable to Lignac-Fanconi disease. Three of the father's siblings (II. 3, 7 and 10) died in childhood of probably unrelated illnesses. II. 14 was a stillbirth. The father's sister (II. 12) and the mother's brother (II. 15) married and have brought up four unaffected children ; a fifth child died in infancy from an apparently unrelated disease. Four other siblings of the father (II. 1, 2, 8 and 11) have had a total of twelve unaffected children. The mother's brother (II. 19) has one normal living child, but there have been four stillbirths.

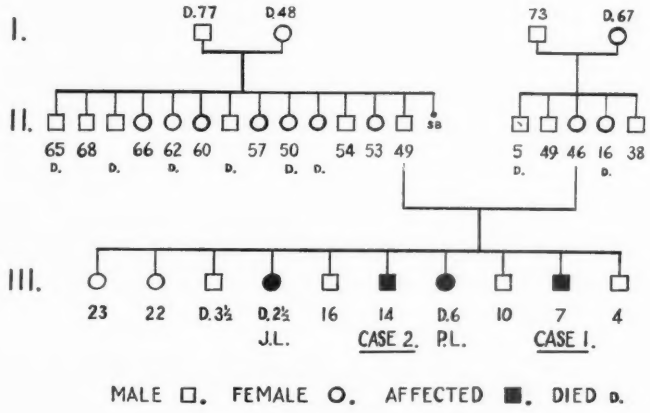


Fig. 1. Family Pedigree (see text).

Fragmentary records of the two dead sisters have been traced :

J.L. Fourth of the ten children, girl aged 2½ in May, 1934, died the day after hospital admission from lobar pneumonia, with post mortem findings of consolidation of right lung and left lower lobe. She had been treated unsuccessfully for rickets since the age of 6 months with cod liver oil and ultra-violet light. She had never walked, and was described as rickety on admission.

P.L. Seventh of the ten children, girl aged 3 in July, 1940, when admitted to hospital. She had never walked, but talked intelligently. Weight was 17½ lbs. (8.0 kg.) ; birth weight 7¼ lbs. (3286 g.) ; breast fed to 1 year ; given cod liver oil regularly. She displayed gross active rickets, with square head and marked widening of epiphyses. Vitamin D concentrates were given with a course of ultra-violet light. Radiographs prior to admission (30.6.40) showed changes characteristic of advanced rickets or renal rickets, with osteoporosis, bowing and separation of the epiphyses (Plate 1). A later film (11.10.40) showed considerable improvement in the condition. though the osteoporosis and the bowing remained. (Films unsuitable for reproduction). Treatment was interrupted under stress of air bombardment (December, 1940) by which time she had gained weight to 22½ lbs. (10.0 kg.). She died at home in 1943 from "pneumonia."

Case 1. K.L., a boy aged 7 years 5 months, was admitted to hospital on 26th September, 1950, for investigation and treatment. His birth weight was 7½ lbs. (3421 g.), and he throve from birth on National Dried Milk. He had always received adequate cod liver oil, and his appetite was good. He had had no serious illness or bowel disorder. He sat up at 8 months, talked "early," stood at 12 months, but did not walk till 2 years. Slowness in growth and curvature of legs were noticed at 3 years of age and ultra-violet ray courses were given until the age of 4. No fractures were ever detected. He had been attending school regularly since the age of 5, making intelligent progress.

Plate 1. P.L. June, 1940.

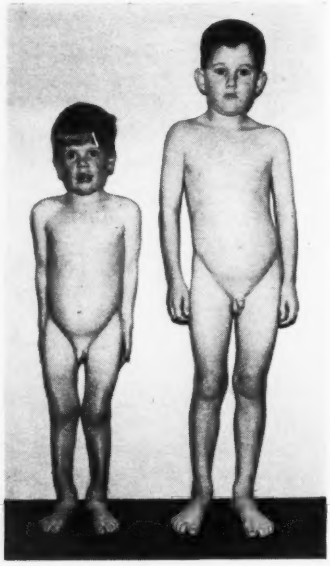


Plate 2. K.L. Sept., 1950, with normal control of same age.

Clinical Examination (Plate 2) : Age 7 years 5 months (26th September, 1950). Growth proportionately stunted. Weight 34½ lbs. (15.7 kg.—Normal : 51½ lbs. or 23.4 kg.). Height 39 ins. (98 cm.—Normal : 47 ins. or 119 cm.). Head circumference 20¼ ins. (51.5 cm.). Normal ruddy complexion and skin texture. Hair normal. Slight rachitic swelling of costo-chondral junctions and of limb epiphyses. Considerable bowing of shafts of both tibiae. No scoliosis or kyphosis. Dentition : formation and development of teeth within normal limits clinically. Ears, nose, mouth, throat and lungs healthy. No lymphadenopathy. No abnormal heart signs. Brachial blood pressure 120/80 mm.Hg. No arterio-sclerosis. Optic fundi : no vascular abnormality, haemorrhage or exudate ; optic discs normal. Slit-lamp examination : definite crystalline deposits in anterior half of corneae. Liver

palpable 1.5 cm. below rib margin. Spleen edge palpable, firm and smooth. No other mass palpable in abdomen. External genitalia normal. No abnormal neurological findings. Muscle development and tone moderate.

Laboratory Investigations (27th September, 1950, onwards) :

Urine : pH range 6 to 8. Protein 320 mgm.%. Benedict's reagent : very strong reduction ; glucose proved by osazone and paper chromatography (Part 3, Fig. 9). Moderate number of hyaline and granular casts and scanty cellular casts, with no excess of cells. Specific gravity throughout the day ranged between 1024 and 1011. Daily output of urine average 24 ounces (710 ml.).

Blood Count (3.10.50) : Haemoglobin 13 g.%, R.B.C. 4,600,000 per cmm., W.B.C. 7,200 per cmm., Platelets 193,000 per cmm.

Blood Chemistry : see Table 1.

Table 1.

Blood levels

	Case 1. K.L.		Case 2. D.L.		
	3.10.50	26.1.51	3.10.50	4.1.51	26.1.51
Plasma Albumin g. per 100 ml.	1.97	2.5	2.16	—	2.9
Plasma Globulin g. per 100 ml.	2.32	1.8	2.2	—	2.7
Plasma Fibrin g. per 100 ml.	0.18	—	0.33	—	—
Serum Calcium mg. per 100 ml.	9.1	9.2	8.0	6.7	6.7
Serum Phosphorus mg. per 100 ml. ..	2.8	4.4	3.2	6.2	6.6
Serum Chlorides (NaCl) mg. per 100 ml.	696	606	705	—	614
Blood Urea mg. per 100 ml.	23	30	74	55	91
Blood Cholesterol mg. per 100 ml. ..	250	—	310	—	—
Thymol Turbidity units	4.0	4.0	3.0	—	3.0
Alkaline Phosphatase units (Jenner and Kay)	40.0	30.0	32.0	—	54.6
Plasma CO_2 vols. %	34.7	37.1	27.1	40	38.0

Glucose Tolerance Curve (20 g. orally) (6.10.50) : Fasting : 101 mg.% ; 20 minutes : 110 mg.% ; 40 minutes : 124 mg.% ; 60 minutes : 128 mg.% ; 120 minutes : 138 mg.% ; 150 minutes : 106 mg.%. **Urea Clearance Test** (16.10.50) with blood urea 18 mg.%, showed standard urea clearances of 96.3% and 72.5% normal function in first and second hours. **Gastric Analysis** (26.10.50) : showed free and total acids within normal ranges.

Bone Marrow Smear (10.10.50) : showed crystals microscopically resembling cystine crystals.

Radiographs of Skeleton (29.9.50 and 6.10.50—see Plates 3 and 4) : Skull : slight widening of the sutures. R. forearm and both legs : marked osteoporosis. Changes at the epiphyseal ends of the bones suggestive of rickets, with considerable

bowing of the bones. No fractures. (No abnormality seen in chest. The spleen appears to be considerably enlarged and there is slight enlargement of the liver.)

Case 2. D.L., aged 14 years 6 months, was discovered through reference of his younger brother K.L. (Case 1) and was admitted to hospital on the same day. He had never been able to attend school by reason of stunting, rickety deformity and inability to walk beyond a few yards. Hospital records of early childhood (age 2-5) were traced, including radiographs of 1939 and 1940, before and after vitamin D administration.

He is the sixth of the ten children (see Fig. 1). Birth weight was 9½ lbs. (4333 g.) and he was breast fed for six months. Subsequently he received cod liver oil "off and on." He sat up at 12 months, walked with support at 18 months, then became weaker, and when admitted to hospital (June 1938) at the age of 2¼, his weight was

Plate 3. K.L. Sept., 1950.

Plate 4. K.L. Sept., 1950.

only 19 lbs. 10 ozs. (8.91 kg.), and he could not stand or crawl. Dentition was not retarded. Marked pigeon deformity of chest with diaphragmatic sulcus and beading of costo-chondral junctions was then present. At the age of 3 (March, 1939) epiphyses of long bones of limbs showed gross enlargement. Serum calcium (age 3¼) was 9.8 mgm.% and phosphorus 3.5 mgm.%. He began to walk at 4 years. No clinical fractures were noted ; but deformity of limbs was the subject of orthopaedic consultation at 4½, when he was reported to be very thirsty, with poor appetite and frequent vomiting. Records of urine studies are not available. Weight increased from 19¾ lbs. (8.98 kg.) at age of 4½ to 28½ lbs. (12.95 kg.) at age 5¾ years, under treatment with vitamin D concentrates and ultra-violet light. Radiographs (9.3.39, age 3) : widening of the ends of the diaphyses of the bones of both legs, with osteoporosis and cupping. There is increased width of the metaphysis and there are pseudofractures in the supracondylar region of the femur on both sides. The appearances are consistent with advanced rickets (see Plate 5).

Nineteen months later (11.10.40) : the radiological appearances of the bones of the legs show considerable improvement. The pseudo-fractures have gone. The metaphysis in all sites is of normal width, and the cupping and irregularity of the diaphyses is no longer visible. All that remains is some osteoporosis and pronounced bowing of the bones. (Films unsuitable for reproduction.)

Ten years later (26.9.50), at 14½ years (Plate 6) D.L. weighed 36 lbs. (16.4 Kg. Normal : 105 lb. or 47.7 kg.) and height was 43 in. (109 cm. Normal : 62 ins. or 159.5 cm.). Head circumference 21 ins. (53.3 cm.). He had sustained three fractures of right lower limb at 7 and 8 years of age. He was walking awkwardly,

Plate 5. D.L. March, 1939.

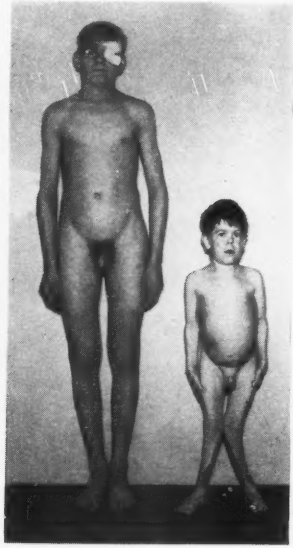


Plate 6. D.L. Sept., 1950, with normal control of same age.

with early exhaustion and breathlessness, and had never been to school. Skin pale and dry. Hair dry and soft. Gross rickety bowing and enlarged ends of long bones of upper and lower limbs. Teeth : formation and development within normal limits, apart from severe alveolar necrosis and sepsis. No pubertal changes in external genitalia (normal size for age). Pubic and axillary hair absent. Heart : no abnormal signs. Blood pressure : 110/70 mm. Hg. Arteries : normal. Optic discs and fundi normal. Slit lamp : definite crystalline deposits in anterior half of corneae. Lungs : no abnormal signs. Kidneys and spleen not palpable. Liver palpable 1 cm. below rib margin. No abnormal neurological findings. Musculature poorly developed and flabby.

Laboratory investigations (27th September, 1950 onwards) :

Urine : pH range 6 to 8. Specific gravity throughout day remained 1013-1014. Protein 270 mgm.%. Benedict's reagent : strong reduction ; glucose proved by osazone and paper chromatography (see Part 3, Fig. 9). Moderate number of hyaline casts, scanty granular casts. No crystals. R.B.C. 3 per high power field. Daily output of urine averaged 36 ounces (1126 ml.).

Blood Count (3.10.50) : Haemoglobin 10 gm.%, R.B.C. 3,600,000 per cmm., W.B.C. 7,800 per cmm., Platelets 248,000 per cmm.

Blood Chemistry : See Table 1.

Glucose Tolerance Curve (20 g. orally) (4.10.50) : Fasting : 94 mg.% ; 20 minutes : 110 mg.% ; 40 minutes : 112 mg.% ; 60 minutes : 154 mg.% ; 90 minutes : 185 mg.% ; 120 minutes : 192 mg.% ; 150 minutes : 127 mg.%. **Urea Clearance Test** (16.10.50) : with blood urea 54 mg.%, standard urea clearances of 32.6% and 44.5% of normal function in first and second hours. **Gastric Analysis** (26.10.50) : free and total acids within normal ranges. **Bone Marrow Smear** (10.10.50) : crystals microscopically resembling cystine crystals (see Plate 7).

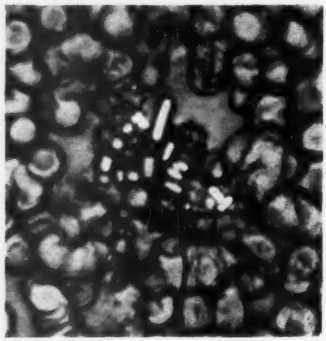


Plate 7. D.L. Sept., 1950. Cystine crystals in sternal marrow (X2050). (Nicol prisms crossed at 45°). K.L. showed similar crystals.

Radiographs of Skeleton (26.9.50—Plates 8 and 9) : All the long bones remain bowed and osteoporotic. There is pronounced forward bowing of the tibia and fibula on both sides with pseudofractures. The pelvis shows tri-radiate deformity with protrusio acetabuli. The appearances at the distal ends of the radius and ulna on both sides are consistent with late rickets. There is still widening of the anterior ends of the ribs.

Special Investigations

Certain additional investigations were made on both K.L. and D.L. The results of these investigations from the two cases are reported together.

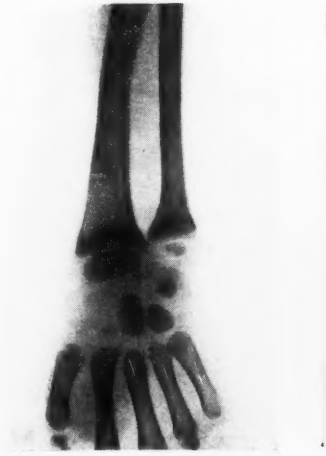


Plate 8. D.L. Sept., 1950.

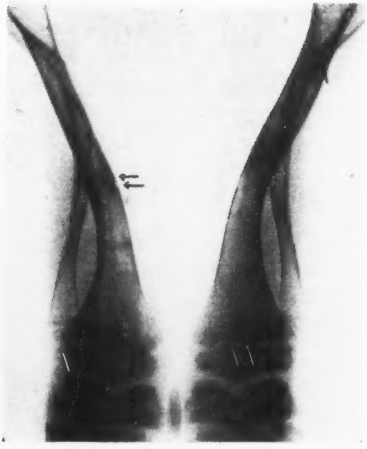


Plate 9. D.L. Sept., 1950.
Arrows show pseudofractures.

Calcium and Phosphorus Balances

The balances were performed on both children over a four day period. A diet containing approximately 1.0 gram of calcium and 1.3 grams of phosphorus was calculated by a dietitian. As it was not possible to keep strictly to this diet, after a preliminary period of three days all food was carefully weighed and charted. At the end of the metabolism period the calcium and phosphorus was recalculated from food tables. A carmine marker was given at the beginning of the four day period. All faeces were saved from the appearance of this marker up to the appearance of a second marker given at the end of the four days. All urine was saved in 24-hour periods. The serum calcium, phosphorus and phosphatase and the blood urea were estimated prior to the metabolism period. Table 2 gives the results of these investigations.

Table 2*a*. Blood levels during Calcium and Phosphorus balances

30.10.50—2.11.50	Case 1 K.L.	Case 2 D.L.	Normal Range
Blood Urea mg. per 100 ml.	18.0	54.0	16—35
Serum Calcium mg. per 100 ml. . . .	9.4	7.8	9.5—10.5
Serum Phosphorus mg. per 100 ml. . . .	2.46	3.9	4.0—4.5
Serum Phosphatase units (Jenner and Kay)	48.0	39.6	6.0—12.0

Table 2b.

Calcium and Phosphorus balances

	Case 1. K.L.		Case 2. D.L.		Normal average for 4-12 years*	
	Ca	P	Ca	P	Ca	P
Calculated average daily intake (g.)	1.021	1.125	0.991	1.081	0.920	1.250
Output in Urine (g.)	0.132	0.480	0.010	0.404	0.096	0.684
Output in Faeces (g.) ..	0.408	0.235	0.492	0.210	0.644	0.393
Total Output (g.)	0.540	0.665	0.502	0.614	0.740	1.077
Retained (g.)	0.481	0.460	0.489	0.467	0.180	0.173
% Total Output :						
In Urine	24%	65%	2%	66%	13%	64%
In Faeces	76%	35%	98%	34%	87%	36%
% Total Intake :						
In Urine	13%	39%	1%	47%	10%	55%
In Faeces	40%	21%	50%	20%	70%	31%

* Macy (1942).

Conclusions

1. At the time of the test both children were in a strongly positive calcium and phosphorus balance. Neither had been receiving vitamin or alkali therapy.

2. The urine calcium output was very low in Case 2 at 2% of the total calcium output ; Case 1 showed a normal output.

3. The urine phosphorus was 65% in Case 1 and 66% in Case 2, these figures being within normal limits.

4. In relation to the intake, the calcium and phosphorus output was low in both urine and faeces. The increased retention may have been due to an increase in storage, which is known to occur on an increased intake, these children coming from poor home conditions.

Serum Electrophoretic Analyses (see Plate 10)

At pH 8.0 in 0.2 molar buffer, protein concentration 2.0%, analyses showed an excess of beta globulin and probably of alpha globulin also in both cases. The albumin and gamma globulin were

within normal limits. (These analyses were kindly made by Dr. NICHOLAS MARTIN, St. George's Hospital, London).

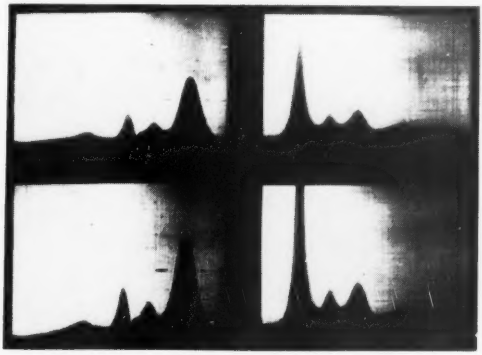


Plate 10. K.L. Dec., 1950. Electrophoretic analysis of serum proteins. D.L. had a similar analysis. (Permission : Dr. N. Martin.)

Urine Aminoacid Investigations

(a) **Paper chromatography** was kindly carried out by Dr. H. BICKEL, the Department of Paediatrics, University of Birmingham, and the result was reported as follows :

The urine of K.L. shows a greatly increased aminoacid excretion (Plate 11). In comparison with the normal aminoacid excretion of children of the same age the increase of glutamic acid, valine, the leucines, proline and lysine is especially noticeable, and, to a lesser degree, that of tyrosine, phenylalanine and threonine. The other aminoacids can be seen in the same concentration in normal urines. The urine chromatogram of D.L. closely resembles that of the brother, K.L., though the concentration of the aminoacids is slightly weaker. There is no excess of cystine in the urine of either child. In further chromatograms of various 24-hr. urine specimens from both brothers the aminoacid pattern remains much the same, though the concentration of the aminoacids varies considerably, sometimes as much as 100%.

(b) **Microbiological Assays :** These, together with assays on the sera of both cases, were kindly performed by Professor H. A. KREBS and members of the Department of Biochemistry, University of Sheffield.

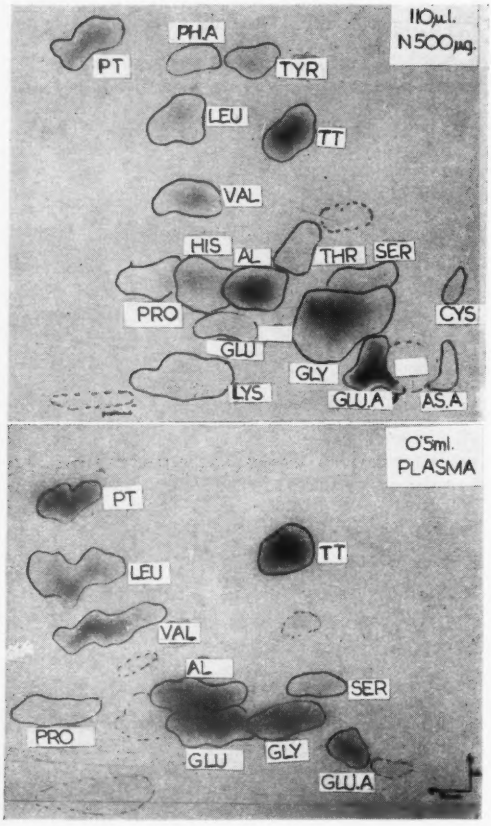


Plate 11 and 12. K.L. Oct., 1950. Paper partition chromatography of urine and plasma. Abbreviations of aminoacid names : TT, test-taurine. PT, test-phenylalanine. For other abbreviations see Fig. 2, Part 1.

Four aminoacids were assayed microbiologically in the urine, using 24-hour specimens collected in bottles containing 15 ml. of chloroform. These were then stored in the refrigerator. The sum of glutamic acid and glutamine was determined by the decarboxylase method (Gale 1945, and Krebs 1948). The sum of glutamic acid, glutamine and histidine was determined by the same technique using Clostridium welchii, strain 6785 of the National Type Culture Collection, and the concentration of histidine was calculated by difference. One or two ml. urine were sufficient for the test.

Cystine and valine were assayed microbiologically in the urine using Lactobacillus arabinosus 17-5 as the test organism. The conditions of growth and the media were essentially as described by Barton-Wright (1946). Response of the test organism was measured turbidimetrically in an E.E.L. nephelometer, usually after incubation for 24-hours.

For the microbiological assay, the urines were freed from urea by treatment with urease as follows : 5 ml. urine were adjusted to pH 5.0 ; 1.0 ml. acetate buffer pH 5.0 and 0.2 ml. urease solution was then added. The mixture was incubated overnight at 40° C., 1.0 ml. 30% trichloracetic acid was added and the precipitate removed by centrifugation. The clear supernatant fluid was extracted twice with an equal volume of ether saturated with water. The ether was removed by aspiration with warm moist air. The sample was then neutralised with caustic soda to pH 6.8 and diluted to a suitable volume for assay.

Acid hydrolysis of the urine was carried out by making the acidity of the sample equivalent to 2N H_2SO_4 by the addition of 50% sulphuric acid and autoclaving for 10 hours at 120° C. The pH was then adjusted to 6.8 by the addition of 5N NaOH and the sample diluted to a suitable volume.

Results of assays (see Table 3) : Twelve samples of urine were tested from each case. Of the four aminoacids assayed, glutamic acid showed the most significant increase in both children compared with ten controls. Cystine appears to be increased in both patients but this may not be significant, as only five control urines were used for this assay compared with twelve from the patients. Histidine was increased in the urine of K.L. but no significant increase was found in D.L.

Table 3.

Microbiological Assays of Aminoacids in Urine

	Range of urine volume per 24 hours	Glutamic Acid*		Histidine		Cystine†	
		Range	Aver.	Range	Aver.	Range	Aver.
Control Patients	270— 930 ml.	23—106	66	25—171	77.2	6—23	12.8
K.L.	343—1000 ml.	172—636	362	82—279	160	13.7—38	27.2
D.L.	450—1230 ml.	128—516	330	0—181	92	14.8—40.6	25.0

* Glutamic acid=Glutamic acid+glutamine.

† Cystine=Cystine+Cysteine.

Aminoacids are expressed as milligrams excreted in twenty-four hours.

Only a few assays of valine have been made but no significant difference between controls and test subjects was found except after acid hydrolysis of the urine, when the latter showed a three to six-fold increase. This, however, may have been due to hydrolysis of residual protein or polypeptides not removed during the preliminary protein precipitation.

Serum Aminoacid Investigations

(a) **The results of paper partition chromatography** were reported by Dr. Bickel as follows : Plasma was deproteinised, desalted but not hydrolysed (see Part 7). The plasma of K.L. shows an aminoacid pattern very similar to that of his urine, with well-defined leucine, valine, proline, alanine, glutamine, glutamic acid and glycine spots (Plate 12). The concentration of aminoacids in this and other specimens is slightly increased. The aminoacid pattern of D.L.'s plasma also conforms to that of his urine. The aminoacid concentration in the plasma shows a slight but definite increase over that of normal plasma. There is no excess of cystine in the blood of either child.

(b) **Microbiological Assays** (Professor Krebs) : Two aminoacids, glutamic acid and cystine, were assayed in the serum of both cases by a method similar to that described for the urine.

The levels for glutamic acid were 15.0 mgm. per 100 ml. in Case 1 and 15.5 mgm. per 100 ml. in Case 2. These figures show a definite increase compared with levels found in healthy adults. (7.6-10.1 mg. per 100 ml.—KREBS, EGGLESTON and HEMS 1949). Unfortunately no data for normal children are available.

In contrast to the glutamic acid, the cystine levels found in the serum from the two cases were low. Both children showed less than 0.3 mgm. per 100 ml., this figure including cystine, cysteine and glutathione. Normal figures for adults found by HUTCHIN et al. (1950) ranged from 1.55 to 2.0 mgm. per 100 ml.

Investigations on other members of the family

Samples of urine from the parents, living siblings and other members from both sides of the family have been investigated by Dr. Bickel for pathological aminoacid and glucose excretion by means of paper chromatography. None of them showed aminoaciduria or glycosuria.

Treatment and Progress

For convenience, the treatment and progress of K.L. and D.L. are reported together. Basically, the treatment in both consisted of the

administration of the citrate-citric acid solution of ALBRIGHT (1940), (sodium citrate 100 grams, citric acid 140 grams, distilled water to 1000 ml.—originally Albright used sodium citrate 98 grams) to combat the low alkali reserve, and large doses of vitamin D in an effort to heal the rickets. Treatment was commenced in both on November 3rd, 1950, but was abandoned three days later as K.L. developed chickenpox. D.L. was reputed never to have suffered from this and both were discharged home temporarily. K.L. recovered from the infection without incident and D.L. did not develop it.

The brothers were readmitted after four weeks and treatment was recommenced on December 8th, 1950. Both received Albright's solution five times a day, starting with 5 ml. doses and increasing each by approximately 5 ml. about every third day according to the rise in the plasma CO_2. This treatment is shown diagrammatically in Fig. 2 which also shows the effect on the plasma CO_2 and urine CO_2. In spite of large doses of alkali a satisfactory plasma CO_2 level could not be reached in either case, although it rose considerably from a minimum of 26 vols.% to a maximum of 40 vols.% in Case 1 and from a minimum of 21 vols.% to a maximum of 48 vols.% in Case 2. The urine CO_2 is shown to have increased enormously with treatment. In both boys a maximum plasma CO_2 level was reached with 50 ml. of Albright's solution daily and this dosage has been continued. In a personal communication, however, Bickel claims that all his patients could be

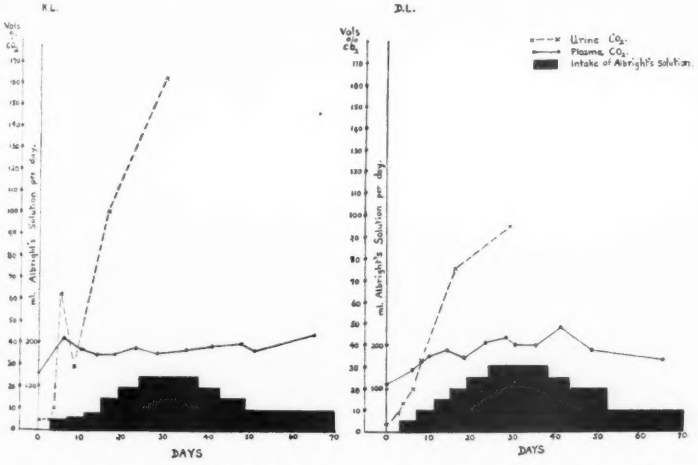


Fig. 2. Relation between alkali therapy, and rise in plasma and urine CO_2.

satisfactorily alkalised, although sometimes only with much higher doses of Albright's solution (120 ml. or more daily).

The same author observed that in six children with the Lignac-Fanconi disease the aminoaciduria ceased gradually some weeks after the beginning of treatment with alkali and that this effect has been maintained for several months (personal communication). Urine and plasma chromatograms were not repeated during treatment in our cases. Microbiological assays, however, were repeated several times on the three aminoacids glutamic acid, histidine and cystine. None of the results showed any significant change, but this may have been due to treatment not having being established long enough and to satisfactorily alkalisation not having been achieved.*

As a high vitamin D intake, both boys were given calciferol 50,000 units daily. On January 4th, 1951, D.L. suffered a major convulsion, lasting five minutes. There were no signs of tetany following the convulsion. Biochemical results obtained on the blood at the time are given in Table 1. In spite of the absence of clinical evidence, the convulsion was presumed to be due to tetany, consequent upon the lowered serum calcium resulting from a rise in the serum phosphorus. This was considered to be a direct result of the high vitamin D intake although the simultaneous alkali administration must also be taken into account. The vitamin D therapy was therefore discontinued, but after eight weeks calciferol 9,000 units daily was recommenced and was continued. Probably an effort should have been made to raise his serum calcium before embarking on alkali therapy. To increase his calcium intake an ounce of cheese (approximate calcium content 250 mgm.) was given daily, being changed to calcium (as lactate) 300 mgm. daily after three weeks. D.L. had no further convulsions. K.L. has continued with the original dosage of calciferol apparently without ill effect.

No other treatment was given to K.L. but D.L. also received ferrous sulphate 9 grains a day for anaemia. In view of his hypotonia and almost complete inability to walk he was given massage, exercises and Faradism to the legs. After three weeks of this treatment he was able to walk up to fifty yards. The general condition of both boys improved greatly, and their weight increased. K.L. weighed 36 lbs. (16.4 kg.) on discharge (27.1.51), an increase of 1½ lbs. (0.7 kg.), while D.L. weighed 41 lbs. (18.6 kg.), an increase of 5 lbs. (2.3 kg.). In

* Since this account was written we have achieved a satisfactory alkalisation in Case 1, at least temporarily. Chromatographic investigation of the urine has been repeated in such a phase and the aminoaciduria is reported by Bickel to have practically disappeared.

appraising the results of treatment it must not be forgotten that these boys were, in all probability, receiving a better and more balanced diet than at home.

After seven weeks of treatment certain investigations were repeated, the results being given in Table 1. X-rays on 27.1.51 showed the following :

K.L. : Considerable improvement in the bones generally. The width of the osteoid tissue at the metaphyses of the femora and tibiae has considerably decreased and is now normal. A similar but not quite so marked improvement has taken place at the lower end of the radius and ulna. Trabeculation has improved, there is less osteo-

Plate 13. K.L. Jan., 1951.

Plate 14. K.L. Jan., 1951.

porosis, and the cortex of the bones generally is practically normal (see Plates 13 and 14).

D.L. : The condition has improved, particularly in regard to the width of the metaphyses, which have considerably decreased. The pseudofractures have healed (see Plates 15 and 16).

Case 2 (D.L.) was readmitted to hospital on March 21st, 1951, having been listless and thirsty for ten days with some shortness of breath. Four days previously he had suffered a series of tetanic episodes. On admission he was extremely ill with signs of left and right sided heart failure. In spite of treatment with oxygen, digoxin

and mersalyl, he died on March 23rd. Just before death his blood urea had risen to 226 mgm.%. Autopsy revealed the changes of acute heart failure. The left ventricle was hypertrophied and the kidneys were contracted. A detailed necropsy report and histological findings are given in Part 8.

Plate 15. D.L. Jan., 1951.

Plate 16. D.L. Jan., 1951.
Note healing of pseudofractures.

Discussion

The gross aminoaciduria, the glycosuria and the skeletal changes identify the cases presented with previously reported cases of Fanconi's syndrome. In addition, cystinosis is present in both boys. This was not proved chemically, but the crystals in the bone-marrow seem to be identical with those described by ESSER (1941) and ROULET (1941). Crystalline deposits were also identified in the cornea of K.L. and D.L., apparently identical with the proven cystine deposits first described by BÜRKI (1941). According to BICKEL (1951) these deposits often cause photophobia, probably due to epithelial defects in the cornea (DOUGLAS, personal communication). There was no photophobia in our cases.

D.L., however, does not entirely conform to the pattern in that he presents hyperphosphataemia with hypocalcaemia, rather than hypophosphataemia, presumably resulting from renal insufficiency. Similar cases have been described previously by various authors,

such as RUSSELL and BARRIE (1936), RÖSSLE (1938) and FANCONI (1946), the children eventually dying of renal failure.

Special Investigations

Both K.L. and D.L. show a gross aminoaciduria. The chromatograms from both compare favourably with those described by Dent in his addendum to the paper of LINDER et al. (1949). We have also been able to show a high excretion of glutamic acid and possibly of histidine by microbiological methods.

No conclusive evidence as to a raised aminoacid blood level has previously been reported, but this may be due to methods not being sufficiently sensitive (FANCONI 1950). Chromatograms of the blood in our cases have shown only a slight increase over the normal, but by microbiological methods a definite increase in the serum glutamic acid has been demonstrated. The possible significance of this will be discussed later.

Electrophoretic analyses of the sera show an increase of beta globulin, and probably some increase of the alpha globulin in both cases, but the significance of this is unknown. FANCONI and BICKEL (1949) observed an increase of alpha globulin and a somewhat smaller increase of beta globulin. The changes may reflect a disordered protein metabolism which is possibly a fundamental element of the disease, but may be secondary to liver damage consequent upon cystine storage in that organ. The low total albumin and globulin levels in the plasma also suggest a liver disorder, for they are probably lower than can be accounted for by the degree of proteinuria. The thymol turbidity, however, was normal in both cases. Cirrhosis and focal necrosis of the liver have been reported in the syndrome on several occasions, both with cystinosis (BEUMER and WEPLER 1937) and without (VAN CREVELD 1934).

Several authors have suggested that the rickets in the syndrome is due to loss of phosphorus through the damaged kidney (FANCONI 1936, MCCUNE et al. 1943, and others). Calcium and phosphorus balances were performed on K.L. and D.L. but excessive phosphaturia was not demonstrated. On K.L. the figures show that the child was in positive balance for calcium and phosphorus. Of the total calcium excreted 24.4% was in the urine, which is within normal limits, but the total calcium excreted is rather lower than the average normal as found by MACY (1942). She showed that in normal children, from the age of 4 to 12 years, on an average daily intake of 920 mg. of calcium (range 823-1197), the average total excretion was 80% of the intake.

Of this 13% was lost in the urine and 87% in the faeces. The increased retention in this patient may have been due to an increased intake, as the hospital diet was probably an improvement on his home diet. LINDER et al. (1949) show a very similar balance on a child of 13 years with hypophosphataemic glycosuric rickets, but she had been treated with vitamin D and citrate-citric acid mixture.

Of the total phosphorus excreted by K.L. 65% was in the urine and 35% in the faeces. These percentages agree with those of Macy, but normal children show a much higher total excretion of phosphorus, usually about 86% of the intake, whilst this child showed only a 59% excretion in the absence of evidence of renal failure.

D.L. also showed a positive balance of calcium and phosphorus but, whilst the percentage of calcium was again higher than normal, the urinary calcium was only 2% of the total excretion. This was due to the low serum level and proves that the hypocalcaemia cannot be the result of a pathological calciuria but is due to another still unclear mechanism. The phosphorus retention was also higher than the average normal, but of the total phosphorus excreted 60% was in the urine, showing good absorption and utilisation.

In neither case can the bony changes be accounted for by excessive phosphorus excretion, as is generally supposed, in spite of the hypophosphataemia in K.L. The cause of the hypophosphataemia is not clear and calcium and phosphorus balances have not assisted in the clarification.

Bickel (personal communication) found in some of his cases of Lignac-Fanconi disease achlorhydria, probably due to degeneration of the oxyntic cells (BAAR 1951). Fractional test meals were performed on K.L. and D.L. but both showed normal total and free hydrochloric acid curves.

Pathogenesis

There is still much doubt and controversy over the pathogenesis of Lignac-Fanconi disease. FANCONI (1931, 1936) first suggested that a renal tubule defect would account for many of the features. Such a defect might cause poor reabsorption of glucose, phosphates, bases and aminoacids, thus producing biochemical disturbances characteristic of the disease. This theory has been taken up by most authors and there is support from the finding of renal changes in many cases at autopsy. The theory has been exploited by STOWERS and DENT (1947) who also found changes in the renal epithelium in their adult case, and demonstrated an absence of phosphatase in the tubule cells.

LINDER et al. (1949) proved poor tubular function in their case by the phthalein excretion test. During treatment we have shown the inability of our cases to conserve alkali, presumably owing to poor reabsorption of base in the tubules. Such a theory, however, does not account for either the generalised cystinosis which occurs in many cases nor the raised aminoacid blood levels which we have demonstrated. Neither does it explain the acetonuria which is often reported.

An alternative theory discussed by FANCONI (1946) is a breakdown in the re-synthesis of aminoacids, these being set free in the blood and excreted in the urine. In certain cases, cystine, where this is in high concentration, might be deposited in the reticuloendothelial system owing to its insolubility. He draws attention to the fact that a high serum aminoacid level would be expected. This theory seems to be more acceptable to us, particularly in view of the fact that a raised glutamic acid level has been found in the sera of our two cases. It is possible that poor tubular reabsorption, with or without demonstrable changes in the renal epithelium, is the result of a nephrotoxic action of amino-acids. Cases living sufficiently long die of renal failure, as in Case 2 (D.L.) in this report, and this might be accounted for in the same way. COX et al. (1929) showed that renal lesions occur in young rats fed on a high cystine diet, and that spontaneous healing occurs when the cystine is discontinued. On the other hand, the renal damage may be due merely to nutritional factors resulting from the aminoacid dysmetabolism.

There are still many queries to answer in the pathogenesis of Lignac-Fanconi disease but to us the primary fault appears to be a defect in the metabolism of aminoacids, as suggested by FANCONI (1946).

Treatment

No form of therapy has been reported as curing the disease or as delaying the onset of eventual renal failure. However, a certain degree of improvement can be obtained in the general condition with various forms of treatment. FANCONI (1936) recommended a diet rich in basic radicles in order to combat the acidosis, but in more recent years alkali in the form of a citrate-citric acid solution, as originally recommended for the treatment of renal acidosis by ALBRIGHT et al. (1940), has been used. This generally results in some rise in the alkali reserve although this was not found by LINDER et al. (1949) in their case. The results obtained by using Albright's solution on our patients have been described above.

Some degree of healing in the skeletal tissues has been shown by various authors using very large doses of vitamin D. MCCUNE et al.

(1943) report complete healing of the rickets in SMYTHE's (1937) case and improvement in that of GUILD (1937). GITTLEMAN and PINCUS (1940) have also reported such improvement. Even with normal doses of the vitamin some healing can be seen and D.L. showed this when first treated for rickets in 1939-40. With 50,000 units of calciferol daily for less than two months both K.L. and D.L. have shown considerable healing of the rickets and some decrease of the osteoporosis. In addition, D.L. who showed hypocalcaemia, had calcium in the form of cheese and later calcium lactate had been given following the tetanic episode. In four weeks of calcium treatment there was no increase in the serum calcium.

FANCONI (1950) warns against the use of excessive vitamin D, as the patient's general condition deteriorated when he used it. VAN CREVELD and ARONS (1949) describe a patient with vitamin D intoxication (three single doses of 300,000 units vitamin D had been given) exhibiting features of Fanconi's syndrome and aminoaciduria. They suggest that vitamin D intoxication may be a causative factor in the disease though little evidence is produced to substantiate this hypothesis. FANCONI and DE CHASTONAY (1950) did not find aminoaciduria in three cases of hypervitaminosis D though they also remark upon the clinical resemblance of such cases to cases of Fanconi's syndrome. In K.L. and D.L. there has been no increase in the excretion of the aminoacids investigated by microbiological or chromatographic methods. The effect of a high dosage of vitamin D must be watched carefully, however, so that toxic signs may be detected early. The increase in D.L.'s blood urea may have been due to this treatment.

With treatment K.L. and D.L. have shown considerable improvement in their general condition. In both the weight increased while in hospital, and they became more active. A further biochemical improvement was shown in their plasma proteins. We consider that these improvements have been effected by treatment with alkalis and vitamin D, but they may be due in part to the wholesome hospital diet. In D.L. marked improvement in his ability to walk was shown, although he is still severely disabled by his deformities. This improvement may have been due partly to the use of physiotherapy.

Genealogy

The occurrence of several cases of Lignac-Fanconi disease in the same family has been noted sufficiently often to suggest that the condition is genetically determined. KAUFMANN (1922) in discussing Abderhalden's case postulated a dominant inheritance, but HANHART

(1940) reviewing the cases of the literature considered it to be transmitted by a recessive gene. FANCONI (1950) came to similar conclusions. STOWERS and DENT (1947) concluded that possibly two allelomorphs may produce Fanconi's syndrome, a dominant gene in adults, and a recessive in children. There is, however, not sufficient evidence that the adult and infantile types of Fanconi's syndrome are related.

In the family of our cases two of ten siblings are affected by the disease and we feel justified in assuming that two others who have died were also affected. The parents deny consanguinity (which was repeatedly recorded by FANCONI in 1950) and no other hereditary factors have been discovered. From the pedigree there seems little reason to doubt that the gene carrying the disease is recessive. There is no record as to the cause of death in childhood of individuals II. 3, 7, 10 and 14. Had they suffered from the disease a dominant gene would have been probable, as it would have required a recessive gene to have affected three of the four individuals in generation I., a possible but improbable occurrence with such a rare gene.

In the pedigree the unique state of affairs exists of a paternal aunt of our cases marrying a maternal uncle (II.12 and 16). None of their four living offsprings have abnormal constituents in the urine but a further offspring died in childhood of an unknown disease. Even had this child died from Lignac-Fanconi disease this would not affect the conclusion that a recessive gene is most probably involved.

Were the disease to show any degree of dominance, then half of the individuals of generation II., left or right (including the parents of the family in question) would be expected to have shown features of the disease. We have no evidence of this whatever. If the character acted as a dominant with incomplete penetrance or a recessive with high penetrance, then the penetrance could not be very low and a 4 : 6 incidence in the family would be unlikely. Also a higher penetrance should have resulted in more symptoms in earlier generations on both sides of the pedigree.

It could be suggested that complementary factors were involved, different dominant genes being contributed by the two sides of the pedigree, each carrying different features of the disease and producing the full disease when combined. Unless the early deaths in generation II had suggested the disease, for which there is no evidence, this is not at all plausible. A dominant mutation would appear to be extremely unlikely.

The incidence of 4 : 6 among the siblings of our cases might appear to be high at first glance. However, in a mating, with the

recessive gene carried by both partners, the chance of a 4 : 6 incidence in a family of ten offsprings is calculated as 0.146 or approximately one in seven.

Summary

The case records of two brothers suffering from Lignac-Fanconi disease are given in detail. Two further siblings probably suffered from the disease. The results of certain special investigations are discussed. In a mineral balance we have not been able to prove that the hypophosphataemia in one of the cases was due to an excessive excretion of phosphorus.

In both brothers the glutamic acid level in the serum was found to be raised. This supports the view that a disorder of aminoacid metabolism is the underlying cause of the disease. It is suggested that the large quantity of aminoacids passed by the kidneys has a nephrotoxic action. The pathogenesis of other features of the disease can be explained by dysfunction of tubular reabsorption caused by this action, and the ultimate onset of renal failure can be explained by the same action involving the glomeruli.

With alkali therapy the general condition of both boys has improved, although a normal plasma CO_2 combining power could not be reached in either case. High vitamin D dosage has produced some healing of the skeletal changes, but attention is drawn to the possibly dangerous effects of this treatment. One case died from heart failure secondary to renal failure after three months treatment.

The genetics of the disease are discussed. The evidence presented from our cases seems to confirm the theory of a recessive inheritance.

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PART 7 : SOME BIOCHEMICAL ASPECTS OF LIGNAC-FANCONI DISEASE

by

H. BICKEL and **E. M. HICKMANS**

from

THE DEPARTMENT OF PAEDIATRICS AND CHILD HEALTH, UNIVERSITY OF BIRMINGHAM, AND THE BIOCHEMICAL DEPARTMENT OF THE CHILDREN'S HOSPITAL, BIRMINGHAM

This paper deals with some biochemical aspects of the calcium-phosphorus, aminoacid, acid-base and sugar metabolism of Lignac-Fanconi disease. The techniques employed and results obtained are described in more detail than in Part 3.

1. PHOSPHORUS AND CALCIUM BALANCES

FANCONI (1936) was the first to suggest that hypophosphataemia and rickets in Lignac-Fanconi disease were due to phosphorus loss in the urine as a result of deficient reabsorption of phosphorus in the proximal tubules. This theory has been accepted by most authors. In nine of our patients, however, calcium and phosphorus estimations in 24-hour urine collections (see Table 3, Part 3) failed to show an increased output of those minerals. Furthermore, therapy with massive doses of vitamin D produced healing of the rickets as in resistant rickets. The question arose whether faulty absorption of calcium and phosphorus from the intestine rather than deficient reabsorption in the kidneys was the principal cause of rickets in Lignac-Fanconi disease. In an attempt to answer this question calcium and phosphorus balances were carried out in a child suffering from the acute form of the disease (Case 1, age 2 years) and in two chronic patients (Case 8, age 6 years, and Case 9, age 9 years). The results were compared with balances recorded by earlier investigators as well as with balances on normal children by MACY (1942). Finally, the balances in Lignac-Fanconi disease were compared with those in resistant and renal rickets.

Methods

Diet and Collection of Specimens. The severe anorexia of our patients made it impracticable to offer a calculated diet. Our practice was to give them a mixed diet which they liked and to send to the laboratory an exact replica for analysis. Food refused or vomited was also analysed and subtracted. In each test the food intake for two periods of three days each was determined and the second period, which in all three tests agreed well with the first, was used for the calculation of the

intake during the balance. At the beginning and end of the second period carmine was given by mouth and the stools collected from the appearance of the first up to the appearance of the second carmine. Urine was collected over the second 3-day period. For Case 1, who was incontinent, a metabolic bed was used. No therapy was given during the balances. No vitamin D was administered for more than three months beforehand except in Case 1, whose daily vitamin doses of 50,000 units were stopped only 3 weeks before the balance and clearly influenced it.

Estimations. Three days' food was mixed and homogenised. A suitable aliquot was removed, ashed in a silica crucible, first over a flame, then in a muffle, dissolved in dilute HCl and finally analysed for calcium (TISDALL and KRAMER 1921) and for phosphorus (BELL and DOISY, modified by BRIGGS 1922). Daily milk specimens were analysed separately. The stools were dried in an open glass dish at moderate temperature to avoid charring. The dried material was weighed, ground up finely and a suitable aliquot removed for estimation by the same methods. The 24-hour collections of urine were shaken well, and aliquots used for estimations.

Results

Mineral balances over periods of a few days are of limited value in obtaining a proper insight into disturbances of several years duration. To reduce the likelihood of error our results were compared with previous balances on patients with the Fanconi syndrome as carried out by FANCONI (1936), GOLDMAN and ECKSTEIN (1939), GITTLEMAN and PINCUS (1940), VAN CREVELD and GRÜNBAUM (1941), MCCUNE et al. (1943), and LINDER et al. (1949). In order to facilitate comparisons the various results of these workers and of our own are summarised in Tables 1 and 2, giving first the absolute values for daily intake and output of phosphorus and calcium, then the percentage of the total mineral output in urine and stool, and finally the percentage of the intake recovered in urine and faeces.

In evaluating the results, the influence on balances No. 4, 10, 11, 14 of massive vitamin D doses given shortly before or during the test should be taken into account, as this explains the good mineral retention and the somewhat atypical mineral distribution in urine and faeces. The correctness of some figures in balance No. 8 was questioned by MCCUNE (1943). Finally the conclusions to be drawn from the balance results depend largely on the definition of a normal balance and pathological values. It is on this distinction that many of the differences of opinion between us and earlier workers are based. We consider that MACY'S (1942) extensive balance studies (593 balances on 29 normal children) provide excellent data for normal children. Her average figures for the four and eight year old groups are given in column 1, Tables 1 and 2.

(a) **Phosphorus balances.** Apart from the balances under vitamin D influence, five of seven balances were negative, i.e. more phosphorus

was excreted than was ingested and there was no retention. This was due to a pathological loss of phosphorus in the faeces. Normally about 29-33 per cent of the total phosphorus output is recovered in the stool, whereas in Lignac-Fanconi disease the output was increased to 50-49-39-90-72-39 and 38 per cent. Accordingly the percentage in the urine was reduced to subnormal figures and no hyperphosphaturia was recorded. The percentage of the phosphorus intake recovered in the faeces was raised in every balance from the normal average of 27 per cent to 50-32-33-106-74-38-61 per cent. In the urine the percentage of the phosphorus intake was found to be reduced in all but McCune's patient (column 13), whose intake, however, was abnormally low (473 mg. per day) due to severe anorexia. This is bound to increase the percentage in the urine, but it is all the more remarkable that even under such starvation conditions the small amount of phosphorus ingested was not properly absorbed : 61 per cent of the phosphorus intake was lost in the faeces. Similar results were obtained by Fanconi (column 7), whose patient ingested only 449 mg. per day, instead of 1000 mg. or more by a healthy child. All the four phosphorus balances carried out under influence of vitamin D were positive, but in three of them the phosphorus loss in the faeces was increased. None of these children developed phosphaturia.

(b) **Calcium balances.** Only two of the seven calcium balances without vitamin D were negative, but two others showed an abnormally low retention of +73 and +67 mg. per day (normal average +170 to +217 mg. per day). As in the case of phosphorus, this decreased retention was due to calcium loss in the faeces. Of the total calcium output the four patients with decreased retention excreted 91-98-95 and 90 per cent in the faeces (normal average 80-82 per cent) and proportionately less than normal in the urine. The percentage of the calcium intake in the faeces was increased and in the urine decreased, except in balance No. 12, which showed a good retention and a normal distribution of calcium in stool and urine.

The balances under vitamin D influence showed a normal or even increased calcium retention of +729, +439, +235 and +425 mg. per day (normal average +170 to +220 mg.). This must have been due to improved absorption, which is also recognisable in the small recovery of the ingested calcium in the faeces of two of these patients (balance No. 4, 50 per cent and No. 14, 36 per cent ; normal 61-65 per cent). In the last patient mild calciuria of 204 mg. per day (normal average 110-160 mg.) was observed, which we also attribute to the high

Table 1.

PHOSPHORUS AND CALCIUM BALANCES in the authors' cases of LIGNAC-FANCONI DISEASE and in RESISTANT and RENAL RICKETS

Balance No.	1	2	3	4	5	6
Author	Macy 1942	Bickel and Hickmans				
Diagnosis	593 balances on 29 normal children	LIGNAC-FANCONI DISEASE			Resistant rickets	Renal rickets
Age	4 and 12 Y. groups	Case 8, M.R., 6 Y.	Case 9, M.B., 9 Y.	Case 1, K.C., 2½ Y.	Sh. H., 6½ Y.	Da. S., 12½ Y.
Weight	18.4—41.8 Kg.	12.2 Kg.	13.5 Kg.	8.3 Kg.	17.3 Kg.	22.3 Kg.
Food, drugs etc. .. (See footnotes)	(*a*)	(*c*)	(*d*)	(*e*)	(*f*)	(*g*)
BLOOD CHEMISTRY						
Serum Ca mg.%	9—11	10.0	5.6	10.5	10.4	8.2
Serum P mg.%	5.5	4.6	7.7	3.3	3.1	8.5
P'tase Units (Kay) ..	8—12	22	26	30	67	23
Urea or N.P.N. mg.% ..	20—40	23	285	29	31	144
PHOSPHORUS BALANCES (DAILY AVERAGES)						
Intake mg.	1141—1501(*b*)	780	558	927(*e*)	1172(*f*)	1162
Output in Urine mg. ..	642—975	394	186	469	453	276
Output in Faeces mg. ..	311—395	387	177	302	596	1062
Total Output mg. ..	953—1370	781	363	771	1049	1338
Balance mg.	+188—+131	—1	+195	+156	+123	—176

% OF OUTPUT AND INTAKE IN URINE AND FAECES						
% Total Output in Urine	67—71%	50%	51%	61%(*e*)	43%(*f*)	21%
% Total Output in Faeces	33—29%	50%	49%	39%	57%	79%
% Intake in Urine ..	56—65%	50%	33%	51%	39%	24%
% Intake in Faeces ..	27—26%	50%	32%	33%	51%	91%
CALCIUM BALANCES (DAILY AVERAGES)						
Intake mg.	829—947	793	501	1324(*e*)	965 (*f*)	1054
Output in Urine mg. ..	109—159	65	29	39	17	69
Output in Faeces mg. ..	503—618	654	319	756	836	1173
Total Output mg.	612—777	719	348	795	853	1242
Balance mg.	+217—+170	+73	+153	+729	+112	—188
% OF OUTPUT AND INTAKE IN URINE AND FAECES						
% Total Output in Urine	18—20%	9%	8%	5%(*e*)	2%(*f*)	6%
% Total Output in Faeces	82—80%	91%	92%	95%	98%	94%
% Intake in Urine ..	13—17%	8%	6%	3%	2%	7%
% Intake in Faeces ..	61—65%	83%	64%	50%	87%	111%

(*a*) (*c*) and (*g*). Complete diet without vitamin D medication.
(*b*) The two figures are average values for the 4 and 12 year groups respectively.
(*d*) Complete diet, but severe anorexia. No vitamin D.
(*e*) Complete diet. 50,000 I.U. calciferol given for 7 days, 3 weeks before beginning the balance.
(*f*) Complete diet. 8,000 I.U. calciferol daily for months until 2 weeks before beginning the balance.

Table 2.

PHOSPHORUS AND CALCIUM BALANCES in probable cases of LIGNAC-FANCONI DISEASE in the literature, compared with balances on normal children

Balance No.	1	7	8	9	10	11	12	13	14
Author	Macy 1942	Fanconi 1936 case 3	Goldman and Ekstein 1939	Gittleman and Pincus 1940		v. Creveld and Grünbaum 1941	McCune et al. 1943		Linder et al. 1949
Diagnosis	593 balances on 29 normal children	Cystinosis	Renal dwarf + glycosuria + rickets	Fanconi syndrome period 1	period 8	Renal rickets + cystinuria	Fanconi syndrome period 1	period 2	Fanconi syndrome
Age	4 and 12 Y. groups	2½ Y.	2 Y.	5 Y.		2¼ Y.	8¾ Y.		13 Y.
Weight	18.4–41.8 Kg.	7.4 Kg.	8.7 Kg.	13.4 Kg.		7 Kg.	12.3 Kg.		22.3 Kg.
Food, drugs, etc. (see footnotes)	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)	(*g*)	(*h*)	(*i*)
BLOOD CHEMISTRY									
Serum Ca mg.%	9—11	10.6	14.1	10.8		11.0	10.5		10
Serum P mg.%	5.5	1.17	1.46	2.0		2.0	2.1		2.1
P'ase Units	8—12	—	—	32		23	42		65
Urea or N.P.N. mg.%	20—40	57	32	—		25	31		18—23
PHOSPHORUS BALANCES (DAILY AVERAGE)									
Intake mg.	1141—1501	449	658	1044	1576(*e*)	989(*f*)	877	473	1272(*i*)
Output in Urine mg.	642—975	233	78	297	737	116	518	458	456
Output in Faeces mg.	311—395	262	699	772	611	543	336	286	328
Total Output mg.	953—1370	495	777	1069	1348	659	854	744	784
Balance mg.	+ 188— + 131	—46	—120	—25	+ 228	+ 330	+ 23	—271	+ 488

Balance mg.	+188—+131	—46	—120	—25	+228	+330	+25	—271	+425

	% OF OUTPUT AND INTAKE IN URINE AND FAECES								
% Total Output in Urine ..	67—71%	47%	10%	28%	55%(*e*)	18%(*f*)	61%	62%	58%(*i*)
% Total Output in Faeces	33—29%	53%	90%	72%	45%	82%	39%	38%	42%
% Intake in Urine ..	56—65%	52%	12%	28%	47%	12%	59%	97%	36%
% Intake in Faeces ..	27—26%	58%	106%	74%	39%	55%	38%	61%	36%
	CALCIUM BALANCES (DAILY AVERAGE)								
Intake mg.	829—947	434	917	1508	1506(*e*)	1280(*f*)	830	745	986(*i*)
Output in Urine mg. ..	109—159	9	18	69	52	12	117	65	204
Output in Faeces mg. ..	503—618	460	750	1468	1015	1033	457	613	357
Total Output mg.	612—777	469	768	1537	1067	1045	574	678	561
Balance mg.	+217—+170	—35	+149	—29	+439	+235	+256	+67	+425
	% OF OUTPUT AND INTAKE IN URINE AND FAECES								
% Total Output in Urine ..	18—20%	2%	2%	5%	5%(*e*)	1%(*f*)	20%	10%	36%(*i*)
% Total Output in Faeces ..	82—80%	98%	98%	95%	95%	99%	80%	90%	64%
% Intake in Urine	13—17%	2%	2%	5%	4%	1%	14%	8%	21%
% Intake in Faeces ..	61—65%	108%	81%	98%	67%	81%	55%	82%	36%

(*a*) (*e*) and (*g*) Complete diet.
(*b*) Complete diet but severe anorexia. Fever before balance.
(*d*) Milk, fruit, and glucose diet.
(*e*) Same diet as (*d*) + daily administration of H_3PO_4 and 6250 U.S.P. units vitamin D for several weeks.
(*f*) Butter-milk+fruit juices. Anorexia before balance. 15—30,000 I.U. vitamin D daily for 3 weeks before beginning the balance.
(*h*) Same as (*g*) but severe anorexia.
(*i*) Neutral ash (no further comment). 10,000 I.U. vitamin D. daily for several months before beginning the balance.

vitamin D intake of 10,000 units daily for months ceasing only shortly before the balance.

(c) **Balances in Lignac-Fanconi disease with hyperphosphataemia and in renal rickets.** Chemical investigations in the later stages of Lignac-Fanconi disease show that hypocalcaemia and hyperphosphataemia may develop, and the blood chemistry then becomes similar to that of classical renal rickets. It is of special interest to analyse balance findings at this stage of the disease in order to try to assess how far they are influenced by the progressive destruction of the kidney. Unfortunately the only source of information so far is the balance No. 3 of our Case 9, who exhibited severe hypocalcaemia and moderate hyperphosphataemia and uraemia. This girl's general condition was poor, she vomited several times during the balance, had an irregular temperature spiking to 100° F. and died two months later. Her phosphorus and calcium intake were reduced to 558 and 501 mg. per day respectively. During the test both calcium and phosphorus balances were positive, but this cannot be regarded as representative of the mineral metabolism of the patient, as X-ray examination revealed severe decalcification of the bones (Part 4). Table 3 shows some relevant phosphorus and calcium data for this patient, compared to a patient with renal rickets and to normal children. Four points stand out :

(1) Hyperphosphataemia of 7.7 and 8.5 mg.% was found in both patients but was more pronounced in renal rickets.

(2) Hypocalcaemia in the patient with Lignac-Fanconi disease (5.6 mg.%) was considerably stronger than in the patient with renal rickets (8.2 mg.%), despite the small increase in the phosphorus blood level.

(3) In the urine, phosphorus and calcium excretion were reduced in both patients. The low phosphorus excretion is especially remarkable in view of the raised phosphorus blood level.

(4) In the stool, phosphorus and calcium were lost in excess by the patient with renal rickets, thus producing a negative balance ; the faecal excretion of calcium (1173 mg. per day) actually exceeded the calcium intake (1054 mg. per day) and the phosphorus excretion (1062 mg. per day) almost equalled the phosphorus intake (1162 mg. per day). The patient with Lignac-Fanconi disease showed a decreased mineral content in the stool, due to poor intake and perhaps to some loss of food in the vomits, the complete collection of which was sometimes difficult.

Table 3.

CALCIUM, PHOSPHORUS BALANCES in LIGNAC-FANCONI DISEASE compared with RENAL RICKETS

	Condition	Serum level mg.%	Daily intake mg.%	Daily output in urine mg.	Daily output in faeces mg.
P	Controls, 4 and 12 years old ..	5.5	1140—1500	640—975	310—395
	Case 9, Lignac-Fanconi disease	7.7	558	186	177
	Renal rickets	8.5	1162	276	1062
Ca	Controls, 4 and 12 years old ..	9—11	830—950	110—160	500—620
	Case 9, Lignac-Fanconi disease	5.6	501	29	319
	Renal rickets	8.2	1054	69	1173

Discussion and Conclusion

Contrary to previous theories, we interpret the balances in the literature and in our patients as evidence that the skeletal lesion in the hypophosphataemic stage of Lignac-Fanconi disease is due to increased loss of calcium and phosphorus in the faeces and not in the urine. Nevertheless, there is probably an increased phosphate clearance in some cases, where the phosphorus excreted in the urine is high in relation to the blood level, which is usually low. This increased clearance may be explained by secondary hyperparathyroidism and has also been described in ordinary vitamin D-deficiency rickets (see Part 8). Evidence of hyperparathyroidism in some of our cases is given in Part 8. Raised phosphate clearance is thus no proof of the renal origin of the rickets.

The loss in the faeces may be due either to decreased intestinal absorption or to increased re-excretion from the blood into the intestinal tract. The latter possibility, accepted by MITCHELL (1930) for renal rickets, is unlikely in Lignac-Fanconi disease, as the serum levels of phosphorus and calcium were low or normal in all but one case where mineral balances were done. We consider, therefore, that the increased mineral loss in the faeces is due to poor absorption. Our balances do not seem to differ in any way from those in vitamin D-resistant rickets, and for comparison a balance in such a patient is given in Table 1, column 5.

Our theory is supported by the fact that under the influence of massive doses of vitamin D four patients showed a satisfactory retention of calcium and phosphorus. This was admirably demonstrated in the studies of GITTLEMAN and PINCUS (1940), who followed the mineral balance of their patient before and during several weeks of treatment with vitamin D. They commented as follows : "The metabolic studies revealed that she was in a positive calcium balance immediately after the administration of vitamin D. The phosphorus balance, however, remained negative up to the fourth period, after which time she retained both calcium and phosphorus." The mineral retention thus achieved leads to healing of the rickets, as demonstrated in Part 4. The phosphorus blood level finally becomes normal, but only after prolonged and successful treatment of rickets and osteoporosis, and after a proper maintenance dose for vitamin D has been established.

The behaviour of the calcium and phosphorus metabolism in the later stages of the disease with hypocalcaemia and hyperphosphataemia cannot yet be fully assessed, as so little data is available. The balances in our Case 9 might lead one to assume that kidney destruction plays an important rôle, as the glomerular lesion leads to reduction of the phosphorus clearance, and thus causes retention of phosphorus in the blood similar to that in renal rickets. A comparison of the two diseases, however, reveals certain differences in their mineral metabolism. In renal rickets the hyperphosphataemia is more marked, partly perhaps because the phosphorus rise starts from normal blood levels. Phosphorus values as high as 16.1, 18.2 and 25.0 mg.% were recorded for renal rickets by several authors (PARSONS 1927, SCHOENTHAL and BURPEE 1930, SCOTT-EASTON and PATERSON 1928). In Lignac-Fanconi disease, on the other hand, no case has, as far as we know, been described with a serum phosphorus level of above 10 mg.%. We conclude that hypocalcaemia in renal rickets is secondary and proportional to hyperphosphataemia, whereas in the later stages of Lignac-Fanconi disease it develops before hyperphosphataemia (Part 3, p. 38) and is due to years of deficient intestinal absorption. The hyperphosphataemia in this disease is less marked, as it starts from a low level, which again is due to poor intestinal absorption.

The course of events in Lignac-Fanconi disease might, therefore, be described as follows : Disturbed intestinal absorption of phosphorus and calcium causes hypophosphataemia and deficient calcification of the skeleton. The calcium level in the blood is kept normal as long as

possible but finally drops to subnormal levels. This initiates a rise in the low phosphorus blood level to normal or somewhat increased values. Finally, as kidney destruction advances, decreased glomerular filtration may lead to reduced phosphorus clearance, thus contributing further to the hyperphosphataemia in the terminal stages of the disease.

2. CHROMATOGRAPHIC AND MICROBIOLOGICAL STUDIES OF THE AMINOACID METABOLISM

The disturbance of the aminoacid metabolism in Lignac-Fanconi disease is by no means limited to the sulphur-containing aminoacids but affects most of the aminoacids to be found in the human body. Our methods of investigation had, therefore, to reveal as many individual aminoacids as possible and to show their behaviour in the plasma and urine. Two-dimensional paper chromatography seemed to meet this demand, and proved most satisfactory in demonstrating the excessive excretion of many aminoacids in the urine of our patients and in comparing the aminoacid pattern in their urine and plasma with those of healthy children and patients suffering from other disturbances of aminoacid metabolism. It does not, however, provide an accurate quantitative basis for the estimation of aminoacids. In this matter we were greatly assisted by Professor KREBS (Sheffield University) to whom we are indebted for carrying out determinations on the plasma and urine of our patients by the glutaminase method (KREBS 1948, 1949), and to Dr. SCHREIER (Heidelberg University) for microbiological assays (SCHREIER and PLÜCKTHUN 1950). There follows an account of the methods used and the results obtained.

Methods

Preservation of specimens. For chromatography, fresh urine specimens were collected with toluene or thymol crystals and kept at 4° C. 24-hour urine collections from patients with Lignac-Fanconi disease are especially liable to decomposition owing to the frequently high urine pH ; they were, therefore, further preserved with chloroform or merthiolate and frequently compared with a fresh specimen of the same day. Blood specimens were heparinised, centrifuged immediately and deproteinised by filtration through collodion sacs (GREENBERG and GUNTHER 1929) in a vessel surrounded by ice. The filtrate was kept at 4° C. Blood and fresh urine specimens were always taken in the post-absorptive state. For microbiological assay heparinised plasma and urine were preserved with toluene after the plasma had been deproteinised by Folin's method with 0.66N H_2SO_4 and 10% sodium tungstate in the ratio 2 : 2 : 1. They were then sent by post to Heidelberg. Unfortunately they were sometimes more than a week in the post and the possibility of changes in their aminoacid content cannot be excluded. Such changes would, however, consist in a decomposition of the aminoacids, and would thus not invalidate the high values recorded by Schreier. For glutamine estimations urine was preserved

with chloroform and heparinised plasma with caprylic alcohol. The specimens were then sent to Sheffield, where they arrived within 24 hours.

Chromatography. Our technique closely resembled the method described by CONSDEN, GORDON and MARTIN (1944), DENT (1947, 1948) and HERMANN, BICKEL and FANCONI (1949). A urine volume containing 500 μg. nitrogen was pipetted on to a sheet of No. 4 Whatman filter paper, unless a 24-hour urine collection was examined, when the volume used was one millionth part of the total volume. For plasma estimations, 0.5 ml. of the heparinised, deproteinised and desalted (CONSDEN, GORDON, MARTIN 1947) specimen was taken. Urine and plasma were treated with ammonium molybdate and hydrogen peroxide on the paper, after which two-dimensional descending chromatography was carried out, employing phenol-water as the first, and collidine-lutidine-water as the second solvent. Sodium cyanide and ammonium hydroxide were added to the phenol box, diethylamine to the collidine box. The temperature of the room was thermostatically controlled at 25° C. After the runs the papers were dried, sprayed with a 0.15% ninhydrin solution and dried again at a temperature not exceeding 40° C. The aminoacid spots thus developed were identified according to their position on the paper. Their colour intensity was recorded by using an arbitrary colour scale (DENT 1947) with ten shades, ranging from 1 for the weakest to 10 for the strongest colour. More recently the colour intensity has been compared with test spots of pure taurine, which were put in five different positions and concentrations (5-10-20-40-60 μg) on the right hand edge of the paper before starting the run. The final positions of these taurine test spots can be seen on some of the chromatograms reproduced in Part 3 (Fig. 4, 6). This procedure was found to be preferable, as the intensity of the final ninhydrin colour reaction varies slightly from chromatogram to chromatogram, which renders a comparison with a fixed colour scale rather unsatisfactory.

The spots developed by ninhydrin nearly always represent free aminoacids and occupy a characteristic position on the paper. Peptides and amines may, however, occasionally give a ninhydrin reaction(DENT 1947, BREMNER and KENTEN 1951). If, therefore, a spot appeared in an atypical position, the specimen was hydrolysed with HCl and the run repeated. The disappearance of the spot was taken as proof that it was due to peptides. Confusion with amines is serious only in a few special areas of the paper, as pointed out by Bremner and Kenten.

Microbiological assay by Dr. Schreier. Schreier has recently published a detailed description of his technique (SCHREIER and PLÜCKTHUN, 1950) which is essentially that of HENDERSON and SNELL (1948). Leuconostoc mesenteroides P-60 was used for the assay of cystine, histidine, leucine, isoleucine, lysine, methionine, serine, tyrosine and valine ; lactobacillus arabinosus 17-5 for tryptophane and phenylalanine ; streptococcus faecalis for arginine and threonine. Differences from Henderson's techniques lay mainly in vitamin additions to the media and in the final estimation of the acid formation, which was performed by measuring the pH of the incubated final medium with a quinhydrone electrode. The results of this method are in accordance with those obtained by ionometric titration (TEERI and JOSSELYN 1949, SCHREIER and PLÜCKTHUN 1950).

Quantitative determination of glutamine and glutamic acid by Professor Krebs. GALE (1945, 1947) and KREBS (1948, 1949) have established that washed suspensions of Clostridium welchii specifically decarboxylate glutamic acid and l-glutamine. The actual quantitative determination of the sum of these aminoacids is carried out

by manometric measurement of the carbon dioxide evolved on decarboxylation. In our patients the sum of glutamine + glutamic acid alone was estimated, as it shows less variation than the concentration of either of the two components. (For details see KREBS 1948, KREBS, EGGLESTON and HEMS 1949).

Results

Chromatography. During the last two years 629 urine and 136 plasma chromatograms of patients with Lignac-Fanconi disease have been carried out. Table 4 gives the colour intensity of urine chromatograms in 12 cases of Lignac-Fanconi disease. For every patient a characteristic chromatogram was chosen and the colour intensity of the spots compared with an arbitrary scale as described above. The normal control figures were obtained by testing the urine of 200 healthy school children and 50 toddlers. Chromatograms of a volume of normal urine containing 500 μg. nitrogen generally show only a few faint aminoacid spots, mainly glycine, alanine, glutamic acid, glutamine, histidine, cysteic acid and taurine (in order of colour strength). The sum of the colour values of the spots rarely exceeds 40-50 arbitrary colour units, or the colour given by 80 μg. taurine. Much the same is true of infants, except in the first days of life, when a definite aminoaciduria is found (Fig. 2, Part 1).

In Lignac-Fanconi disease, the pattern was distinctly different from that of the normal urine chromatogram, with a general increase of some 10-20 aminoacids, especially the leucines, valine, lysine, proline, serine, cystine, aspartic acid, tyrosine, phenylalanine and threonine and also of the aminoacids found in normal urine, with the exception of histidine. Cystine was oxidised to cysteic acid, as it does not otherwise give a ninhydrin reaction. The total colour intensity in different patients ranged from values just above normal (66) to values considerably over 100 (Table 4).

In reading the chromatograms, it must be borne in mind that the aminoacids show a varying sensitivity to ninhydrin. Phenylalanine, tyrosine and proline, for instance, have a considerably smaller ninhydrin sensitivity than glycine, valine, taurine, etc. The colour intensity of the spots does not, therefore, always accurately reflect their concentration. This is one of the main reasons why quantitative evaluation of chromatograms has so far been unsatisfactory.

Plasma chromatograms of 9 patients with Lignac-Fanconi disease are summarised in Table 5 and are compared with normal plasma taken from 30 healthy volunteers.

In most normal plasma chromatograms well marked aminoacid spots were produced by alanine, glutamine, glycine, valine, the leucines,

Table 4.

CHROMATOGRAPHIC FINDINGS in URINE of 12 cases of LIGNAC-FANCONI DISEASE, expressed in units of an arbitrary colour scale, 1 for the weakest, >10 for the strongest colour

	Normal		Cases												
	Aver.	Range	1 K.C.	2 P.R.	5 J.N.	6 D.S.	7 O.R.	8 M.R.	9 M.B.	10 K.L.	11 D.L.	12 A.M.	13 M.L.	14 J.S.	Average
Alanine	2.7	0—6	>10	≫10	>10	>10	10	≫10	>10	8	>10	10	≫10	≫10	>10
α-amino-n-butyric acid ..	0	0	0	0	0	5	0	1	1	0	0	2	0	0	7.5
Arginine	0	0	0	7	0	2	2	5	7	0	0	0	2	0	2
Aspartic acid	0.3	0—3	10	>10	9	9	0	2	4	3	4	0	2	5	5
β-alanine or citrulline ..	0	0	9	9	2	0	0	0	8	0	0	4	0	0	3.5
Cystine as cysteic acid ..	0.6	0—4	10	>10	10	8	7	6	8	5	1	6	1	5	7
Glutamine	1.8	0—7	≫10	10	6	5	8	≫10	>10	6	5	≫10	≫10	8	>10
Glutamic acid	3	0—8	>10	≫10	10	10	4	10	8	>10	>10	9	>10	5	>10
Glycine	6	0—10	8	>10	10	10	10	≫10	9	>10	10	8	≫10	>10	>10
Histidine	2.2	0—7	4	4	2	0	7	5	3	7	1	2	2	2	3
Leucine and iso-leucine ..	0.1	0—2	≫10	>10	10	9	2	8	9	8	6	10	≫10	8	>10
Lysine	0.05	0—1	10	>10	3	7	2	6	9	7	4	8	>10	8	8
Methionine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phenylalanine	0	0	6	7	0	2	1	4	0	1	1	3	6	0	2.5
Proline and hydroxyproline	0	0	+	++	++	—	—	+	+	++	++	+	++	+	+
Serine	0.5	0—5	8	>10	9	1	5	10	8	6	3	5	5	5	7
Taurine	1.1	0—6	0	0	0	0	5	0	0	0	0	0	0	0	0.5
Threonine	0	0	2	9	0	3	0	4	5	2	0	2	1	0	2.5
Tyrosine	0.2	0—2	8	9	1	4	1	6	2	4	1	3	2	6	4
Valine	0.2	0—4	≫10	≫10	10	10	2	9	10	7	8	10	>10	10	>10
Total	18.7	0—65	>125	>145	>92	>95	66	>106	>111	>84	>64	>92	>91	>82	>113.5

Volume of urine used ≡ 500 μg.N.

glutamic acid, proline and serine (in order of colour strength) ; less constant and fainter spots by lysine, cystine, taurine and histidine ; whereas spots of tyrosine, phenylalanine, arginine and threonine were seen only occasionally. The total colour intensity rarely exceeded 60-70. In patients with Lignac-Fanconi disease the pattern of the plasma chromatograms does not show any striking deviation from the normal or any excessive increase of one or two special aminoacids, as for instance in phenylketonuria, where the phenylalanine spot is very strong. An accurate quantitative comparison of the colour spots in these chromatograms with those of normals is difficult, as the variations of the aminoacid levels in plasma are very much smaller than in the urine of patients with Lignac-Fanconi disease, where a tenfold increase of various aminoacids is common. We nevertheless felt that in several plasma specimens the colour intensity of valine, the leucines, proline, alanine, glutamine, serine, aspartic acid, lysine and β-alanine and/or citrulline, perhaps also of tyrosine, was above that to be expected in normal plasma. There was no definite increase in cystine (as cysteic acid). The sum of the values for all the spots exceeded 80 in 6 of the 9 specimens. An increase in the plasma level of various amino-acids was thus indicated, but, owing to the semi-quantitative nature of paper chromatography, not proved.

Microbiological assay. The results on 24-hr. urine specimens of Cases 1, 2 and 8 are given in Table 6. For purposes of comparison the first column shows normal values, as obtained by Schreier in children of various age groups (SCHREIER and PLÜCKTHUN 1950a, b, and personal communication). Nearly all the normal figures are maximal levels and were not exceeded by any of the healthy infants and children investigated. Normal urine levels for adults have been published by several authors (STEELE et al. 1947, ECKHARDT and DAVIDSON 1948, SCHEFFNER et al. 1948, WOODSON et al. 1948, HARVEY and HARWITT 1949), who unfortunately differ considerably from each other and from Schreier on this point. This is probably due to such factors as age and food intake of the subjects tested, differences in the technique of the assay, etc. Even so, most of the aminoacid values in the urine of our patients were considerably higher than the highest normal values recorded by these workers.

Compared with Schreier's normal values our three patients' urine showed an up to twentyfold increase of valine, leucine, methionine and tryptophane. The excretion of tyrosine, phenylalanine and isoleucine was increased up to ten times, that of threonine, lysine, cystine and

Volume of urine used = 500 µg.N.

Table 5.

CHROMATOGRAPHIC FINDINGS in PLASMA of 9 cases of LIGNAC-FANCONI DISEASE, expressed in units of an arbitrary colour scale, 1 for the weakest, >10 for the strongest colour

	Normal		Cases									
	Aver.	Range	1 K.C.	2 P.R.	5 J.N.	6 D.S.	8 M.R.	9 M.B.	10 K.L.	11 D.L.	13 M.L.	Average
Alanine	8	4—10	10	10	8	>10	>10	>10	10	10	>10	>10
α-amino-n-butyric acid	0.2	0—2	0	0	0	0	0	0	1	1	0	0.2
Arginine	0.7	0—4	0	0	0	0	0	3	0	1	0	0.4
Aspartic acid	0	0	5	>10	2	10	3	0	1	6	0	5
β-alanine or citrulline	0	0	0	6	2	6	2	8	2	1	0	3
Cystine as cysteic acid	1	0—6	1	3	7	3	1	7	0	0	0	2.5
Glutamine	8	2—10	8	10	6	8	>10	>10	>10	10	10	>10
Glutamic acid	4	1—6	9	>10	9	>10	10	7	8	10	6	10
Glycine	7	3—10	6	7	8	10	10	9	8	>10	10	9
Histidine	1	0—7	2	0	0	6	1	0	0	1	0	1
Leucine and iso-leucine	5	2—7	8	>10	9	8	5	10	8	9	10	9
Lysine	2	0—7	3	9	2	6	3	7	6	5	2	5
Methionine	0	0	0	1	0	0	0	0	0	0	0	0.1
Phenylalanine	0.3	0—3	2	8	0	2	0	1	0	1	1	2
Proline and hydroxyproline	+	— or +	+	++	—	+	+	+	++	++	—	+
Serine	3	0—6	6	10	6	9	8	7	5	7	3	7
Taurine	0.7	0—2	3	0	6	6	4	1	1	1	1	2.5
Threonine	0.2	0—2	1	9	0	1	0	1	0	1	0	1.5
Tyrosine	0.2	0—2	2	7	0	4	0	2	0	1	0	2
Valine	6	3—9	8	10	10	9	7	>10	7	8	8	9
Total	52	28—78	74	>120	75	>108	>74	>93	>67	>83	>61	>89

The plasma was deproteinised, volume used 0.5 ml. The normal values are based on a study of plasma from 30 healthy students.

Table 6.

AMINOACID EXCRETION in URINE in LIGNAC-FANCONI DISEASE
expressed as mg. per 24 hrs.
(Microbiological assay by Dr. Schreier)

	Normal children*	Cases 1. K.C.		2. P.R.	8. M.R.	
Arginine	8—15	57	65	69	35	72
Cystine	app. 50	app. 270	app. 190	app. 380	app. 150	app. 220
Histidine	up to app. 120	138	170	164	140	115
Iso-leucine ..	up to 5	52	18	37	18	32
Leucine	up to 5	108	149	99	68	70
Lysine	up to 20	92	115	57	46	81
Methionine ..	app. 1	16	36	18	8	19
Phenylalanine ..	up to 8	72	78	37	36	29
Threonine ..	app. 10	53	64	45	28	29
Tryptophane ..	up to 5	95	83	80	52	61
Tyrosine	up to 5	68	37	33	18	61
Valine	up to 5	140	46	108	85	31

* Schreier 1950 and personal communication.

arginine up to five times. The histidine excretion was normal or slightly raised. Only these 12 aminoacids were estimated.

The results of microbiological assay on deproteinised plasma of Cases 1, 2, 5 and 8 are given in Table 7. Schreier's normal values accord well with average values from other laboratories as reviewed recently by KREBS (1950), except for methionine, for which Schreier's value is lower than that quoted by Krebs. For cystine no normal value was given by Krebs.

Compared with the normal values our patients showed an up to 100 per cent increase in the plasma of tryptophane, tyrosine, phenylalanine, leucine, isoleucine, cystine and methionine. The levels of

The plasma was deproteinised, volume used 0.5 ml. The normal values are based on a study of plasma from 30 healthy students.

threonine, valine, lysine and arginine were raised up to 50 per cent, the level of histidine was normal in 3 of 5 estimations.

Table 7.

AMINOACID PLASMA LEVELS in LIGNAC-FANCONI DISEASE
expressed as mg. per 100 ml.
(Microbiological assay by Dr. Schreier)

	Normal Children average*	Cases 1. K.C.		2. P.R.		5. J.N.	8. M. R.
Arginine	2.4	2.9	3.6	3.1			
Cystine	2.0	3.1	3.5	4.0	2.1	2.7	2.7
Histidine ..	1.7	1.7	3.5	1.7		1.5	2.2
Iso-leucine ..	1.5—1.6	2.1	3.0	2.3	2.4	3.2	3.0
Leucine	2.1	3.5	5.4	3.8	4.1	6.8	4.0
Lysine	3.0	4.3	5.6	4.1	3.9	4.4	4.1
Methionine ..	0.3—0.4	0.6	0.6	0.5	0.65	1.2	0.8
Phenylalanine ..	1.6	2.6	3.3	3.0			
Threonine ..	2.2	3.7	2.8	3.6	3.4		
Tryptophane ..	1.1	1.9	2.4	2.4	2.1	2.3	1.9
Tyrosine	1.6		1.1		3.8	3.2	5.0
Valine	3.0	4.2		5.0			

* Schreier 1950 and personal communication.

Glutamine and glutamic acid estimations. The findings in plasma and urine of Cases 1, 2 and 8 are given in Table 8. Compared with the normal daily glutamine excretion in ten children with unrelated diseases (KREBS, unpublished, personal communication), our patients showed an up to tenfold increase of glutamine plus glutamic acid in the urine.

The normal plasma levels given in the table originate from healthy adults (KREBS et al. 1949) ; it is unlikely that they differ to any degree

from children of the age of our patients. Compared with normal levels, three glutamine+glutamic acid estimations in the plasma of our patients showed an up to 100 per cent increased plasma level. Four further estimations were carried out on specimens taken during periods when the patients were under alkali therapy with sodium citrate and showed no aminoaciduria in urine chromatograms. They all showed normal plasma levels.

Table 8.

GLUTAMINE + GLUTAMIC ACID ESTIMATIONS in PLASMA and URINE of 3 patients with LIGNAC-FANCONI DISEASE

Case	Date	Glutamine + Glutamic Acid	
		Plasma mg.%	Urine mg./24 hrs.
Normal		7 – 10(1)	23 – 106(2) aver. 66
1	1.11.50		443
	26. 2.51		940
	15. 4.51	8.3(3)	
	29. 5.51	13.3	556
2	15.11.50		388
	15. 4.51	8.4	
	18. 5.51	17.5	210
	24. 5.51	22.3	266
8	15.11.50		245
	26. 2.51		49(3)
	15. 4.51	8.3(3)	
	23. 5.51	8.1(3)	(12.3mg.%)(3)

(1) Krebs, H. A. ; Eggleton, L. V. ; Hems, D. ; Biochem, J. ; 1949, **44**, 159.
(2) Unpublished findings of Krebs, H. A., in 10 children with various unrelated diseases (personal communication).
(3) Under alkali therapy, with normal plasma CO_2 combining power and normal urine chromatogram.

Discussion

The results obtained by three different methods confirm earlier chromatographic findings of a substantial and generalised aminoaciduria in Lignac-Fanconi disease (FANCONI and BICKEL 1949, D'AVIGNON and VAHLQUIST 1949). Up to 20 aminoacids, essential and unessential, are involved, but in contrast to various other aminoacidurias, histidine and taurine are usually in normal concentration.

There has been a discussion as to whether this aminoaciduria is due primarily to disturbed reabsorption of the aminoacids in the tubules or to an overflow from an increased blood level into the urine. The latter explanation now appears to be the more probable, as the plasma levels of the aminoacids excreted in excess in the urine are 50-100 per cent above normal. If the tubular defect be the primary factor in this disorder, we have no satisfactory explanation of either the raised aminoacid plasma level or the cystine storage, which was a constant finding in all our patients and has repeatedly been observed in the early stages of the disease during the first and second years of life (HOTTINGER 1947, DRABLØS 1951 and AUTHORS' observations). It remains to be seen by future clearance work if the rise in the aminoacid blood level is high enough to account by itself for the strong aminoaciduria or if a disturbance of the tubular reabsorption is a contributing factor, perhaps as a result of the advancing kidney destruction in the course of this disease, or due to a generalised enzyme deficiency affecting the kidney as well as other organs.

It is unlikely that the actual aminoacid loss in the urine is an important factor in the disease. If compared with the daily intake, the aminoacid loss seems negligible. For example, in one of our acutely ill patients, aged 2 years, the aminoacids excreted in greatest strength were leucine (149 mg. per day) and valine (140 mg. per day). This patient consumed in his daily milk alone about 4 g. leucine and 3 g. valine. ROSE's (1949) figures of the minimum daily requirement for man of these two aminoacids are 1.1 and 0.8 g. respectively. After having subtracted the aminoacid loss in the urine from the intake, a sufficient amount of leucine and valine should, therefore, be available to satisfy the metabolic requirements of this patient, and the same is true of the other essential aminoacids.

In view of the disturbed intestinal absorption of calcium and phosphorus in Lignac-Fanconi disease, one might speculate as to whether there is also a similar disturbance of the absorption of aminoacids. This, however, seems not to be the case, as D'AVIGNON and VAHLQUIST (1949) found that in a tolerance test their patient had a normal absorption curve for casein hydrolysate. Paper chromatograms of stool ultrafiltrates in Case 1 in this series gave the same aminoacid pattern as stool filtrate of a healthy control on a similar diet suggesting that there is no disturbance of the absorption of one particular aminoacid. Moreoever, a deficient intestinal absorption of aminoacids could not explain the high aminoacid level in blood and urine.

Our present knowledge of the aminoacid disturbance in Lignac-Fanconi disease supports the assumption of a generalised disturbance of the aminoacid metabolism, which may take place with or without active participation by the kidney. The disorder does not seem to concern deamination, as urea and ammonia formation are usually undisturbed. Further research into protein synthesis might throw more light on the site of the defect and for this purpose tracer work may be useful.

3. ELECTROLYTE STUDIES IN LIGNAC-FANCONI DISEASE

The nature of the acidosis in Lignac-Fanconi disease differs from that seen in any other condition, although some findings recall the mechanism of certain known acidoses. Thus ketosis, as seen in undernourishment and diabetes mellitus, has been demonstrated in the urine and plasma of various patients with Lignac-Fanconi disease (GITTLEMAN and PINCUS, 1940 and MCCUNE, MASON and CLARKE, 1943). The majority of our patients occasionally showed ketone bodies in their urine, though the ketonuria was often slight or completely absent, despite pronounced acidosis in the plasma. Ketosis cannot, therefore, be an important factor in the acidosis of the disease.

In 1936 FANCONI discussed the possibility of a disturbed renal reabsorption of bases as the cause of the acidosis. This question has gained added importance since a similar tubular dysfunction was postulated as the cause of "renal acidosis," first described by LIGHTWOOD (1934, 1935) and recently investigated in detail by LATNER and BURNARD (1950). LINDER, BULL and GRAYCE (1949) have studied the acid-base metabolism in a probable case of Lignac-Fanconi disease and have shown how complex is the disturbance.

Our investigations were based on the assumption that the acidosis is prerenal in origin, like the disturbance of the aminoacid and calcium-phosphorus metabolism. Renal dysfunction is in our opinion a secondary development, the result of progressive kidney destruction. Any research into the nature of the acidosis in this disease had, therefore, to take into account the extrarenal as well as the renal component, and had to cover the whole electrolyte structure of plasma and urine.

Experimental. The urine electrolytes of one patient suffering from the acute form of Lignac-Fanconi disease (Case 1, age 3 years) and one suffering from the chronic form (Case 8, age 7 years) were investigated while having their usual mixed diet and without any medication. They were then given 200 ml. and 300 ml. respectively of 0.1 N HCl daily by mouth to determine their ability to acidify the urine,

increase the ammonia and reduce the bicarbonate excretions. Their uncertain appetite made it difficult to maintain a constant diet during this period. Later on, on a determined intake of food, more complete acid-base balances were undertaken in Case 1, and on a normal control of the same age, both being on their ordinary diets without medication. This was followed by a period when both were given $CaCl_2$ by mouth, 1 g. daily during one week and 2 g. daily during a second week. The level of the blood electrolytes during these periods was also determined.

(a) **Collection of specimens.** Since Case 1 and the control child were still incontinent, they were kept on metabolic beds. The 24-hr. urine specimens were collected without loss under toluene and preserved with merthiolate. Because in the first test our work had shown that estimations of ammonia and bicarbonate made on fresh specimens usually tallied well with those made on 24-hr. collections preserved as described above, in the second series, ammonia and bicarbonate were estimated exclusively on fresh specimens collected at about the same time each day under toluene—these volumes of course being included in the 24-hr. volumes. Blood specimens were taken in the post-absorptive state in tubes containing liquid paraffin and heparin. They were centrifuged and the plasma taken off at once.

(b) **Diet.** During both tests Case 1 was given his ordinary diet, which consisted mainly of citrated milk ($1\frac{1}{2}$ pints or more daily) with bread, butter, jam, tomatoes, greens, minced meat and occasionally an egg. In the second test, replicas of the food offered during 3-day periods were estimated for sodium, potassium and chloride. The values of any food left over and also of any vomit were subtracted. Case 8 and the control child were given diets similar to that given to Case 1 but with less milk. The intake by the control child of the various elements was determined in the same way as that of Case 1.

(c) **Estimations** on the plasma, the food and the urine during these tests were based on the well-known methods for sodium (NOYENS 1939), calcium (TISDALL and KRAMER 1921), phosphorus (BENEDICT and THEISS 1924), and chloride (CLAUDIUS 1922). Potassium was estimated by flame photometer. We are indebted to Drs. BARCLAY and IBRAHIM, University of Birmingham, for carrying out numerous potassium estimations. Magnesium was not estimated, but ordinarily accepted values were used in the calculations. Bicarbonate estimations in the plasma and fresh urine were made by VAN SLYKE and NEILL's method (1924). Ammonia in the urine was estimated by the method of VAN SLYKE and CULLEN (1916). Inorganic sulphate in the urine was estimated by the method of FISKE (1921); it was not estimated in plasma, but the average normal level of 1 mEq/l was used in the calculation of the acid-base balance. Titratable acidity of the urine was estimated by the method of HENDERSON and PALMER (1914). Protein in the plasma was estimated by a micro-Kjeldahl digestion followed by steam distillation. Since the titration method of VAN SLYKE and PALMER (1920) for organic acids in urine is fallacious in the presence of aminoaciduria (DENT, personal communication), their levels were calculated by the indirect method of subtracting the known anions from the total cations. The same indirect method was used for blood. pH was read on a Cambridge glass electrode potentiometer.

Results

(a) **Electrolyte structure of plasma on ordinary diet without medication** (Tables 9 and 11). Compared with figures usually accepted as standards (shown in Table 9) and also with our figures for the normal control (shown in Table 11), Case 1 exhibited on 3 occasions a hypoelectrolytaemia due to a decrease in the sodium level, and he showed also a decreased potassium level in 3 out of 4 estimations. The chronic Case 8, on the other hand, showed a normal total electrolyte and sodium level, but the potassium level was low in both estimations, reaching on one occasion a value of only 1.8 mEq/l. Of the anions the bicarbonate of Case 1 was reduced in all four specimens, while it was normal in Case 8. The chlorides were always below normal or at the lower limit of normal in both children. The phosphate level was always reduced. The normal level of organic acids in plasma has not yet been established and estimates of the upper level are given in the literature as 0, 3.8 and 13 mEq/l (LINDER 1949) ; these discrepancies may be due partly to the unsatisfactory method of estimating organic acids by subtraction of the known anions from the total cations. If we place the upper limit at about 8-10 mEq/l, then this level was exceeded in each case in at least one estimation with 12.3 and 15.2 mEq/l, whereas most of the other values were at the upper limit of normal.

(b) **The electrolyte structure of urine under the same conditions** (Tables 10 and 12). Compared with Macy's figures for normal children of similar ages and the results in our normal control, both children, but especially the acidosed Case 1, lost an abnormal amount of electrolytes in the urine. The 24-hr. urine volume of 1600-3200 ml. was considerably above normal and in its variations roughly parallel to the excess of ions excreted. Case 1 lost excessive amounts of sodium and potassium and the same was true of Case 8, though to a lesser degree. The output of sodium, potassium and chloride is, however, strongly influenced by their intake, which must be borne in mind when assessing the output of these ions. In our first electrolyte study (Table 10) no actual determinations of sodium, potassium and chloride were carried out, though it was calculated that their intake lay considerably below the intake of normal children in both Cases 1 and 8, due to their anorexia. In the second study (Table 12) sodium, potassium and chloride were determined throughout the test in 3-day food collections in Case 1 and in the control child. During the first two 3-day periods without acid feeding Case 1 ingested 4.4 and 4.0 g. sodium, 6.5 and 5.7 g. potassium, 7.2 and 6.9 g. chloride. The intake of the control child was 6.0 and 6.8 g. sodium, 6.9 and 7.3 g. potassium,

Table 9.

PLASMA ELECTROLYTES in 2 cases of LIGNAC-FANCONI DISEASE, under ordinary conditions. Expressed as mEq/l.

	Normal child		Case 1.	K.C.	Case 8.	M.R.
	Average	Range	31.8.51	3.9.51	31.8.51	3.9.51
CATIONS						
Na ..	141	137 —145	130	130	142	142
K ..	4	3.8— 5.0	2.3	3.2	1.8	2.1
Ca ..	5	4.5— 5.8	5.1	4.8	5.1	4.8
*Mg ..	3		3	3	3	3
Total Cations	153		141	141	152	152
ANIONS						
HCO_3 ..	25	21.4— 28.0	18.2	14.5	28.1	27.0
Cl ..	103	96 —106	97.5	98.7	96.3	90.7
HPO_4 ..	3	2.3— 3.5	1.3	1.8	1.5	2.1
*SO_4 ..	1		1	1	1	1
Org. acids(1)	?4	?0 — 8	6.0	8.2	8.3	15.2
Protein ..	17	14.6— 18.2	16.8	16.6	16.4	16.3
Total Anions	153		141	141	152	152

* No actual estimations made ; average normal values taken.
(1) Indirect estimation by subtracting known anions from cations.

8.8 and 10.3 g. chloride. Macy's healthy children (4 y. group) ingested an average of 6.3 g. sodium, 7.8 g. potassium and 10.2 g. chloride during 3 days. Case 1 thus showed a considerably lower sodium and chloride intake than our control child and a lower sodium, potassium and chloride intake than Macy's healthy children. His potassium intake was relatively high due to his greater milk intake but still insufficient to account for the 100 per cent increased potassium loss in the urine. Ammonia excretion was low in Case 8 on 2 days, and normal on the third occasion. In Case 1, who was severely acidosed and should have shown a high ammonia output, this was only slightly above normal. Titratable acidity in Case 1 was zero, or very small, as the pH of the urine varied from 6.6 to 8.1. In Case 8 the titratable acidity was normal or low and the urine was acid (5.6 to 6.3). Case 1 lost an abnormally high amount of bicarbonate in the urine in spite of his low plasma bicarbonate range of 14.5—9.7 mEq/l. This

Table 10.

URINE ELECTROLYTES in 2 cases of LIGNAC-FANCONI DISEASE under ordinary conditions. Expressed as mEq/Kg/24 hrs.

	Average in normal children (Macy 1942)		Case 1. K.C. age 3 yrs. Wt.=10 Kg.			Case 8. M.R. age 8 yrs. Wt.=12.4 Kg.		
	4 yrs. group. Wt.=18.4 Kg.	8 yrs. group. Wt.=26.2 Kg.	29-30 Aug.	30-31 Aug.	2-3 Sept.	29-30 Aug.	30-31 Aug.	2-3 Sept.
CATIONS								
Fixed bases								
Na	4.2	3.8	7.3	10.1	6.1	4.8	6.5	5.5
K	3.0	2.3	6.8	7.5	7.4	3.8	4.8	3.9
Ca	0.30	0.23	0.24	0.35	0.32	0.28	0.31	0.23
Mg	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Total fixed base	7.7	6.6	14.6	18.2	14.1	9.2	11.9	9.9
Base economy								
NH_4	0.90	0.83	1.5	1.4	2.8	0.34	0.48	0.90
Titr. acidity	0.5 (app.)	0.5 (app.)	0	0	0.40	0.53	0.33	0.38
Total base	9.2	7.9	16.1	19.6	17.3	10.1	12.7	11.2
ANIONS								
HCO_3	0	0	4.1	4.4	2.2	0.08	0.35	0.55
Cl	4.4	3.6	6.4	7.8	7.3	5.1	7.5	5.8
HPO_4	2.0	1.7	1.9	1.7	1.4	1.1	0.92	1.3
SO_4	1.8	1.6	1.6	3.0	1.6	1.1	2.3	1.3
Org. acids	1	1	2.1	2.7	4.8	2.7	1.6	2.2
Total anions	9.2	7.9	16.1	19.6	17.3	10.1	12.7	11.2
Total ions	18.4	15.8	32.2	39.2	34.6	20.2	25.4	22.4
pH of urine			7.41	7.36	6.60	5.60	6.25	6.28
24-hr. volume of urine in ml. ..			2000	2760	3240	1600	2220	1760

accounts for his very high urine pH, up to 8.14, during acidosis. The bicarbonate excretion in the urine of Case 8, who was not acidosed and had a urine pH of 5.6—6.3, was normal. The chloride excretion was raised in all but one specimen in Case 1 and in one out of three specimens in Case 8. Organic acids were excreted in excess in both cases in every instance, sometimes up to 5 times, at other times at least twice the normal value.

(c) **Electrolyte changes in plasma under feeding of 1-2 g. $CaCl_2$ daily** (Table 11). In the healthy control child, this small amount of acid failed to produce any considerable change and did not alter the bicarbonate content. In Case 1, on the other hand, the same dose of $CaCl_2$ reduced the already low bicarbonate levels of 20 and 16 mEq/l to 7 mEq/l, whereupon the test was interrupted as the child became increasingly upset. The other electrolytes were little influenced, except potassium, which dropped from low normal values to very low values of 2.2 and 2.1 mEq/l. On two occasions during the feeding of $CaCl_2$, the organic acid excretion reached the highest observed value of 16.8 mEq/l.

(d) **Electrolyte changes in urine under feeding of 1-2 g. $CaCl_2$ daily** (Table 12). After beginning this feeding, the healthy control child excreted slightly more chloride. The ammonia excretion rose slowly but definitely and to a lesser degree the titratable acidity, while the pH fell from 6.64 and 6.02 to 5.12. There was also a moderate rise of the sulphate excretion. None of these changes, however, was very striking. The child's appetite, electrolyte intake and well-being were unimpaired. Case 1, on the other hand, lost his appetite, so that his sodium, potassium and chloride intake dropped considerably from 4.0, 5.7 and 6.9 g. in the previous 3-day period to 2.8, 3.6 and 4.8 g. respectively. This decreased electrolyte intake was maintained throughout the acid feeding period. The low intake was reflected in the decreased output of these ions. When, however, the $CaCl_2$ was doubled, the extra chloride ions were excreted in increased amount with fixed bases. This may explain the drop of the potassium level in the plasma. The loss of bicarbonate in the urine ceased and the urine reached its lowest pH of 5.38. The titratable acidity increased only moderately. The organic acid excretion increased to its highest level of 4.6 mEq/Kg/24 hrs. in the last two specimens. The ammonia formation, normally the principal and most adaptable mechanism in a state of acidosis, failed to respond properly.

The response of the ammonia and bicarbonate excretion to acid feeding and their influence on the urine reaction were also seen in the first test on Cases 1 and 8 (Fig. 1), when Case 1 reacted in much the

Table 11.

PLASMA ELECTROLYTES in a case of LIGNAC-FANCONI DISEASE compared with a normal control, under ordinary conditions and during $CaCl_2$ feeding. Expressed as mEq/l.

	Case 1, K.C., age 3 Yrs.						Normal Control, P.T., age 3 Yrs.					
	Ordinary Diet		Same diet with lg.$CaCl_2$		Same diet with 2g.$CaCl_2$		Ordinary Diet		Same diet with lg.$CaCl_2$		Same diet with 2g.$CaCl_2$	
	30.10.51	1.11.51	5.11.51	8.11.51	13.11.51	16.11.51	30.10.51	1.11.51	5.11.51	8.11.51	13.11.51	16.11.51
CATIONS												
Na	141	124	127	121	126	136	147	141	141	133	139	132
K	3.7	4.3	3.5	3.8	2.2	2.1	4.3	4.1	4.6	5.0	6.1	6.0
Ca	5.6	4.8	5.3	5.2	5.4	5.0	4.6	4.9	5.1	5.0	5.6	5.0
*Mg	3	3	3	3	3	3	3	3	3	3	3	3
Total Cations ..	153.3	136.1	138.8	133.0	136.6	146.1	158.9	153.0	153.7	146.0	153.7	146.0
ANIONS												
HCO_3	19.7	16.2	13.9	16.2	11.2	7.3	21.9	22.5	21.9	22.9	22.7	22.8
Cl	95.5	85.7	84.6	86.3	94.6	100	108	108	105	101	100	101
HPO_4	1.9	1.8	1.7	1.2	1.3	1.8	2.4	2.4	2.4	2.6	3.0	2.4
*SO_4	1	1	1	1	1	1	1	1	1	1	1	1
Org. acids(1) ..	12.3	8.5	16.8	7.7	8.9	16.8	7.6	1.0	4.9	0	8.0	0.5
Protein	22.9	22.9	20.8	20.6	19.6	19.2	18.0	18.1	18.5	18.5	19.0	18.3
Total Anions ..	153.3	136.1	138.8	133.0	136.6	146.1	158.9	153.0	153.7	146.0	153.7	146.0

Only heparinised plasma in the postabsorptive state was used.
* No actual estimations made ; average normal values taken.
(1) Indirect estimation by subtracting known anions from cations.

Table 12.

URINE ELECTROLYTES in a case of LIGNAC-FANCONI DISEASE compared with a normal control, under ordinary conditions, and during $CaCl_2$ feeding. Expressed as mEq/Kg/24 hrs.

	Case 1, K.C., age 3 yrs. Wt. = 10 Kg.						Normal Control, P.T., age 3 yrs. Wt. = 15 Kg.				
	Ordinary diet		Same diet + 1g. $CaCl_2$ daily		Same diet + 2g. $CaCl_2$ daily		Ordinary diet		Same diet + 1g. $CaCl_2$ daily		Same diet + 2g. $CaCl_2$ daily
	30-31 Oct.	1-2 Nov.	4-5 Nov.	8-9 Nov.	13-14 Nov.	16-17 Nov.	30-31 Oct.	1-2 Nov.	4-5 Nov.	8-9 Nov.	13-14 Nov.
CATIONS											
Fixed Bases											
Na*	6.6	7.1	5.5	5.8	6.8	8.5	4.3	4.5	5.6	4.8	4.5
K*	6.8	7.2	5.5	6.1	6.7	6.4	3.6	2.8	3.0	2.7	2.8
Ca	0.18	0.22	0.15	0.22	0.28	0.25	0.27	0.20	0.14	0.39	0.15
Mg	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Total fixed base	13.9	14.8	11.5	12.4	14.1	15.5	8.5	7.8	9.0	8.2	7.3
Base economy											
NH_4	2.2	1.3	0.71	1.8	1.0	2.3	0.75	0.80	0.84	1.7	2.5
Titr. acidity	0	0	0.31	0	1.2	0.67	0.33	0.60	0.76	0.88	0.91
Total base	16.1	16.1	12.5	14.2	16.3	18.5	9.6	9.2	10 6	10.8	10.7
ANIONS											
HCO_3	3.5	3.2	0.34	1.1	0	0	0	0	0.08	0.03	0
Cl*	6.8	5.9	4.6	6.8	9.9	12.1	5.4	5.6	7.5	6.4	6.9
HPO_4	2.5	2.0	1.8	0.92	1.0	0.65	2.6	2.3	1.7	1.7	1.5
SO_4	1.1	1.3	1.6	1.7	0.8	1.1	0.94	0.93	1.1	2.0	1.5
Org. acids	2.2	3.7	4.2	3.7	4.6	4.6	0.7	0.4	0.2	0.7	0.8
Total anions	16.1	16.1	12.5	14.2	16.3	18.5	9.6	9.2	10.6	10.8	10.7
Total ions	32.2	32.2	25.0	25.4	32.6	37.0	19.2	18.4	21.2	21.6	21.4
pH of urine	8.14	7.38	6.40	7.46	5.40	5.38	6.64	6.02	5.59	6.45	5.12
24 hr. volume of urine in ml.	2050	2160	1700	2250	2300	2400	450	490	530	640	470
Creatinine mg. in 24 hrs.	240	214	244	252	259	226	369	372	371	426	382

* Intake of Na, K and Cl see text.

same way as in the second test. The chronic Case 8 showed practically no increase of the ammonia production under acids, although the plasma bicarbonate dropped from normal levels to 16.9 mEq/l and the urine pH from 6.3 to 4.8.

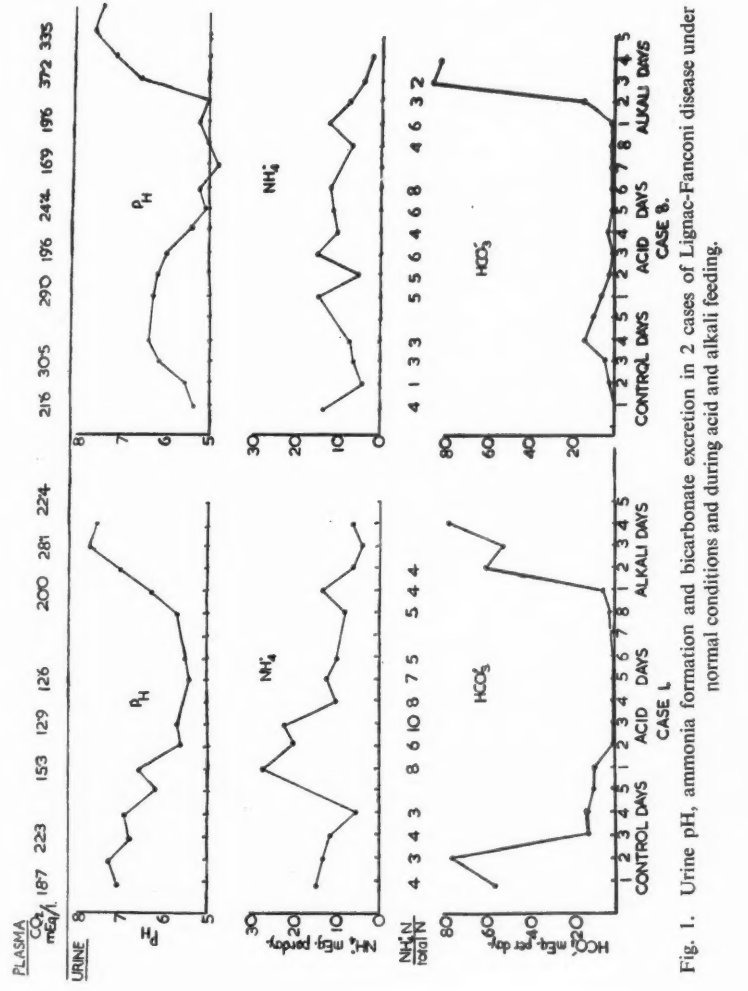


Fig. 1. Urine pH, ammonia formation and bicarbonate excretion in 2 cases of Lignac-Fanconi disease under normal conditions and during acid and alkali feeding.

Discussion and Conclusion

Our investigations have not as yet revealed any simple explanation of the acidosis in Lignac-Fanconi disease. Several factors seem to play a part, namely the increase of organic acids in urine and probably in blood, insufficient ammonia production by the kidney, the loss of the fixed bases sodium and potassium, and faulty reabsorption of bicarbonate, which explains the high pH of the urine. In some cases there was also ketosis. The relative importance of these various factors cannot yet be assessed. Not all patients fail to produce sufficient ammonia ; in fact, some form particularly large quantities, as was stressed by FANCONI (1936, 1946). The loss of bicarbonate through the kidney is certainly an important factor and was observed by Professor KREBS in Philpott's two cases (Cases 10 and 11 of this series, see Part 6) and in our Cases 1 and 2. It is not, however, common to all patients. During acidosis five of our patients excreted acid urine with a pH of less than 6, which excludes any considerable loss of bicarbonate in the urine (see GAMBLE, 1947, chart 24). On the other hand, if such a loss of bicarbonate occurs, it is pathological, as all the bicarbonate is usually reabsorbed by the kidney as soon as the plasma level is reduced (PALMER and VAN SLYKE, 1917, PITTS and LOTSPEICH 1946). GAMBLE (1947) writes : "Base as bicarbonate in the urine, under circumstances demanding conservation of base, must be regarded as base wasted." The bicarbonate must be excreted with ammonia or, if ammonia production is insufficient, with fixed bases. The cause of the bicarbonate loss is still problematic. LATNER and BURNARD (1950) found that the disturbed bicarbonate reabsorption in cases of "renal acidosis" can be corrected by intravenous phosphate infusion. On the other hand, the bicarbonate reabsorption in Case 1 was completely normalised by the administration of hydrochloric acid, which at the same time reduced the urine pH to 5.3.

The loss of fixed bases may be partly due to inadequate ammonia production, as they must then cover the excretion of various acid radicles such as bicarbonate, chloride and organic acids. A loss of potassium is, moreover, to be expected where the protein breakdown is excessive, and is therefore particularly marked in Case 1, where serious disturbance of protein metabolism is demonstrated by the continuous massive aminoaciduria. The increased excretion of sodium and potassium is associated with a moderate loss of chloride.

Finally, the increase of organic acids in urine and probably in blood is of special interest. LINDER (1949) found a similar increase using the same method. For reasons discussed in Part 3 these organic

acids do not represent aminoacids to more than a negligible fraction. Nor were they keto acids in our two patients, as the nitrɔ-prusside test in the urine was negative. The various organic acids involved may in future be identified by new chromatographic methods (LUGG and OVERELL, 1947). We suggest that the origin of the organic acid excess lies in disturbed transamination. Another indication of the disturbance of protein metabolism is the repeated clinical observation that the urine of some patients has a pungent faecal smell (LIGNAC 1924, Case 2, VAN CREVELD and GRÜNBAUM, 1941, our Case 5). This suggests the presence of unidentified amines or "ptomaines" (van Creveld).

Some important clinical considerations may be derived from the preceding observations. The kidneys and prerenal tissues of patients with Lignac-Fanconi disease seem to have partly lost their function of maintaining the isoionia of the plasma. This causes hypopotassaemia, acidosis and dehydration and renders the patient unable to withstand any further acid load during infection, etc. The foregoing observations provide a theoretical justification for alkali therapy with sodium and potassium and for the maintenance of a state of good hydration.

4. THE GLUCOSE SHOCK IN LIGNAC-FANCONI DISEASE AND ITS RELATION TO POTASSIUM

Severe and sudden peripheral collapses during sugar tolerance tests have been observed repeatedly in this disease (DEBRÉ et al. 1934, FANCONI 1936, 1946, FANCONI and BICKEL 1949) but have not so far been satistactorily explained. We have abandoned the hypothesis put forward by FANCONI in 1936 and 1946, that they were the result of hyperglycaemia and acidosis following the sugar ingestion. The blood sugar in most instances was not high enough to justify a diagnosis of hyperglycaemic shock (Part 3, p. 61) nor was there any apparent increase in the blood acidosis during the test, as shown by half-hourly estimations of the CO_2-combining power in three of our tolerance tests ; and in no test did any ketonuria develop.

In view of the importance of hypopotassaemia in this disease and of the similarity between the glucose shock and acute states of hypopotassaemia, it occurred to us that glucose administration might sometimes lead to a dangerous fall in the already low plasma potassium level. The close relationship between the sugar and potassium metabolism has been established by numerous publications in recent years. WELLER and TAYLOR (1950) have made a critical survey of the subject. Glucose will retard the catabolism of protein and so prevent

the release of potassium from the cells in the fasting patient. It also promotes the uptake of potassium from extra-cellular fluid by formation of glycogen and perhaps by restoring certain cellular functions. Cells lose potassium if their supply of glucose is exhausted. For these reasons glucose is recommended as an antidote to hyperpotassaemia by DARROW and PRATT (1950). The inter-relationship between glucose and potassium is, however, mutual : not only is glucose necessary for potassium metabolism, but potassium is vital to cellular glucose metabolism. Liver slices in a high potassium medium form more glycogen than do liver slices in a low potassium medium.

We have studied the effect of glucose ingestion on the potassium plasma level in three glucose tolerance tests in Case 2 and in a healthy child.

Heparinised blood was taken from the neck-vein fasting and then at half-hourly or hourly intervals after oral doses of 30 g. glucose in the first test, 40g. in the second, and 50g. in the third. After blood had been removed for estimation of sugar the remainder was carefully centrifuged for 10 minutes and the plasma used for flame photometer estimations of potassium which were kindly carried out by Dr. Barclay. At the same time in tests 2 and 3 the potassium and sugar excretion before and after the administration of sugar was estimated in two-hourly urine specimens. The same procedure had been carried out on the previous day without giving glucose to provide comparative values showing normal daily variations. On another day a large dose of water was given instead of glucose to observe the effect of diuresis only on the potassium plasma level. Finally the same tests were carried out on a healthy control child of about the same age.

The tests on our patient after 40 and 50 g. glucose are summarised in Fig. 2. They showed the following results :

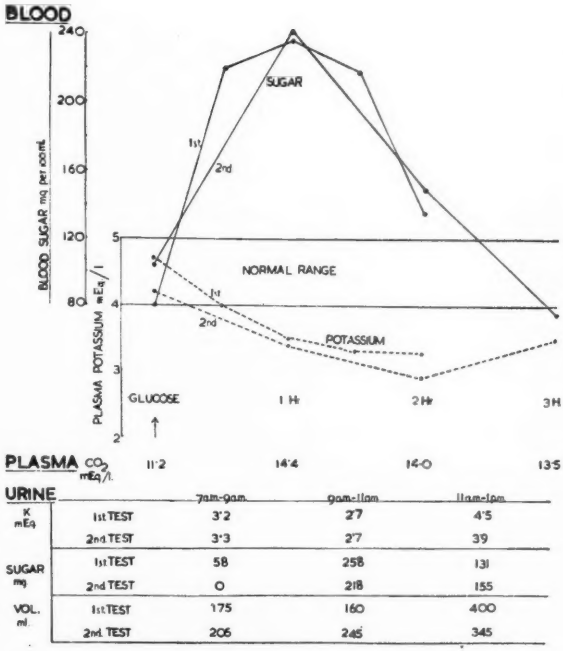
(a) The sugar ingestion caused a fall in the potassium plasma level of 1.4 mEq/l. The lowest potassium level of 2.8 mEq/l was reached after 2 hours, the highest blood sugar level of 240 mg.% after 1 hour.

(b) A similar but slighter and less prolonged fall in the potassium level was seen in the healthy child. The lowest potassium level of 3.7 mEq/l was reached after only 1 hour.

(c) No fall in the potassium level was seen either on the control day or after the dose of water.

(d) Whereas during the first sugar tolerance test the increased potassium excretion in the urine was perhaps sufficient to account for the fall in the potassium plasma level, this was not so in the second test.

The sugar tolerance test thus results in hypopotassaemia, which is apparently not only due to loss of potassium in the urine but also to potassium uptake by the cells. In a mild form this seems to occur even



URINE

		7am-9am	9am-11am	11am-1pm
K mEq	1st TEST	3·2	2·7	4·5
	2nd TEST	3·3	2·7	3·9
SUGAR mg	1st TEST	58	258	131
	2nd TEST	0	218	155
VOL. ml.	1st TEST	175	160	400
	2nd. TEST	206	245	345

Fig. 2. The correlation of blood sugar and plasma potassium during two sugar tolerance tests on Case 2.

in a healthy child, but is not so pronounced or prolonged as in Lignac-Fanconi disease. A "glucose shock" was presumably not observed in Case 2 because the fasting potassium values in the plasma were normal, viz. 4.7 and 4.3 mEq/l, and because the child was clinically in a good phase. If, on the other hand, the potassium fasting level had been decreased to 3 mEq/l or less, as it often is in Lignac-Fanconi disease, then a further reduction after sugar might have resulted in a dangerous hypopotassaemic crisis. We put forward the hypothesis that the so-called "glucose shock" in Lignac-Fanconi disease is really a "hypopotassaemic shock."

SUMMARY

1. Phosphorus and calcium balances in three patients of this series are analysed and compared with eight balances in the literature. Four of the eleven balances were carried out under vitamin D influence

and were positive. Of the remaining seven, five phosphorus and two calcium balances were negative. This was due to phosphorus and calcium loss in the faeces ; neither hyperphosphaturia nor hypercalciuria was detected. The balances in Lignac-Fanconi disease were identical with a balance performed in a case of resistant rickets. Compared with renal rickets the hypocalcaemic, hyper-or normo-phosphataemic form of Lignac-Fanconi disease shows certain differences. Though the excretion of phosphorus and calcium in the urine was decreased in both conditions, the hypocalcaemia in Lignac-Fanconi disease was more marked and the hyperphosphataemia mild or absent. It is suggested that the mechanism of these biochemical changes is different from that in renal rickets.

2. Chromatographic studies of urine and plasma in patients with Lignac-Fanconi disease showed a moderate to strong increase of 10-20 aminoacids in the urine, while in the plasma the aminoacid pattern was normal though the concentration of the aminoacids seemed to be above the normal range. Microbiological assay of twelve aminoacids by Dr. Schreier showed up to a twentyfold increase of various aminoacids in the urine and up to 100 per cent increase in the plasma. Estimations of glutamine and glutamic acid by Professor Krebs showed similar results. These findings do not indicate primarily a renal mechanism of the aminoaciduria, but suggest a prerenal disturbance of the aminoacid metabolism.

3. Electrolyte studies in two patients under normal conditions and after acid feeding point to a complex disturbance of the acid-base metabolism. Several factors seem to be involved, namely increase of organic acids in urine and probably in blood, insufficient ammonia production by the kidney, loss of the fixed bases sodium and potassium, and faulty reabsorption of bicarbonate. The relative importance of these factors is discussed.

4. No satisfactory explanation of the "glucose shock" in Lignac-Fanconi disease has so far been offered. During three glucose tolerance tests we followed the potassium level in the plasma of our patient and of a healthy control. Glucose ingestion resulted in pronounced and prolonged hypopotassaemia in our patient, which was not due to potassium loss in the urine. In the control the response was similar but milder and briefer. We suggest that the "glucose shock" in Lignac-Fanconi disease is a "hypopotassaemic shock."

PART 8: MORBID ANATOMY, HISTOLOGY AND PATHOGENESIS OF LIGNAC-FANCONI DISEASE

(Cystine Storage Disease with Aminoaciduria)

by

H. S. BAAR and **H. BICKEL**

from

THE PATHOLOGY DEPARTMENT OF THE CHILDREN'S HOSPITAL, BIRMINGHAM, AND THE DEPARTMENT OF PAEDIATRICS AND CHILD HEALTH, UNIVERSITY OF BIRMINGHAM

Although the finding of cystine crystals in tissues was briefly mentioned by KAUFMANN in 1922 the credit for establishing a disease characterised by deposits of crystalline cystine in a variety of organs goes to LIGNAC (1924). A few years later FANCONI (1931, 1936), DE TONI (1933) and DEBRÉ et al. (1934) described independently a clinical syndrome in childhood which included albuminuria, glycosuria, acidosis and hypophosphataemic rickets. Only recently has it been realised that the clinical syndrome known as that of Fanconi-de Toni-Debré is closely related to the morbid anatomical entity which was clearly outlined by the studies of LIGNAC (1924, 1926a and b) and of RUSSELL and BARRIE (1936) and to which the names cystinosis or cystine storage disease have been attached.

In 1937 BEUMER and WEPLER suggested that the Fanconi syndrome might be the clinical manifestation of cystinosis, but in 1943 MCCUNE and his associates still believed that cystinosis represented an independent process within the Fanconi syndrome and that the latter represented "one phase, or aspect, of a larger morbid species and merges without perceptible demarcation on the one side with classic hyperphosphataemic renal rickets and on the other with the poorly understood process which is currently called cystine rickets." It would seem reasonable to differentiate cystinosis from the much wider clinical syndrome of renal glycosuria, albuminuria and hypophosphataemic rickets, when one compares the spectacular deposits of cystine in the tissues of some patients with the reported absence of cystine in the tissues of children suffering from the Fanconi syndrome. Fanconi's discovery of aminoaciduria including cystinuria in the syndrome which bears his name, followed by the demonstration of cystine crystals in aspirated bone-marrow by ESSER (1941), FANCONI (1946) and FANCONI and BICKEL (1949), and in the conjunctiva and cornea by BÜRKI (1941) and by ULLRICH (1948), were the first indications

that the association of the Fanconi syndrome with cystinosis is more common than previously believed. Eventually in 1946 FANCONI put forward the thesis that his syndrome and cystinosis are identical diseases.

The presence of cystine crystals in tissues, so conspicuous in some cases, has certainly been overlooked in others. This is particularly clear from the report of RÖSSLE (1938), whose case is now generally accepted as an example of cystinosis. After he had discovered crystalline material in paraffin sections he intended to elucidate their chemical nature. But the crystals had meanwhile "disappeared from the spare material preserved in formalin and from sections embedded in gelatine." We had a similar experience with paraffin sections from kidneys which we had the opportunity to examine through the kindness of Professor Dorothy Russell. This may account for some examples of the Fanconi syndrome in which no cystine storage was found. Some gaps in the pathological reports have doubtless arisen from failure to realise the almost constant and probably obligatory association of cystinosis with the Fanconi syndrome in childhood, and also from the fact that few pathologists have had the opportunity of examining more than one case. We have been able to examine organs from five clear-cut cases of cystinosis with the clinical syndrome of Fanconi-de Toni-Debré and from one case which we believe to be an example of the same disorder, although crystals were found only in aspirated bone marrow and not in the post mortem material. In addition we had an opportunity to examine aspirated bone-marrow from fourteen living patients, biopsy specimens from the conjunctiva of one case, a lymph node biopsy from one case, and kidneys from three cases of Professor Russell as well as from two cases shown to us by Dr. Zollinger in Zürich. On the basis of these findings an attempt will be made to outline the natural history of Lignac-Fanconi disease, to describe methods for the demonstration and identification of cystine crystals in tissues, including bone-marrow films, to show that in doubtful cases a lymph node biopsy will probably always give a definite answer, and to make suggestions concerning the pathogenesis of this disorder.

PATHOLOGIC-ANATOMICAL FINDINGS IN SIX CASES

The following are the gross anatomical and histological findings in six cases of Lignac-Fanconi disease. The numbering of Cases 4, 7, 9 and 11 corresponds to that in the previous parts. The clinical details of Cases 15 and 16 will be published by Dr. R. J. K. BROWN (1952) who has kindly given us permission to include a brief summary

in this paper. For further clinical details of Case 16 since Dr. Brown's completion of his manuscript we are indebted to Dr. O. P. GRAY.

We are greatly indebted for the gross anatomical findings and material for histological study of Case 4 to Dr. W. Whitelaw and Dr. A. E. Chaplin ; of Case 7 to Professor A. V. Neale and Dr. O. C. Lloyd ; of Case 11 to Dr. T. E. Emery ; of Case 15 to Professor J. Gough and Dr. L. L. R. White ; of Case 16 to Professor J. Gough and Dr. R. A. Parker.

Case 4

R.C., a boy who died at the age of 2 years, 11 months, and on whom a post mortem examination was performed approximately 36 hours after death by Dr. A. E. Chaplin. The body was that of a wasted, pale, male child. The trachea and bronchi were normal. The lungs showed slight oedema at both bases. The right side of the heart was dilated ; no structural abnormality was present ; the muscle was pale and toxic. The thyroid was normal in size ; there was no obvious enlargement of the parathyroid glands. The liver was normal in size and showed severe toxic change. The spleen was normal in size ; its pulp was extremely pale. Both kidneys were reduced in size. There was irregularity of the surface. The cortices and pyramids showed linear markings which were yellow in colour. The suprarenals were healthy. The stomach contained some altered blood. Small submucous haemorrhages were present. The small and large intestines showed marked injection and there was oedema in the bowel wall. The brain was oedematous. The venous sinuses were healthy.

Only formalin-fixed material was available for histological examination.

Kidney : The majority of the glomeruli are large, cellular and contain in their capillaries only few red blood corpuscles. The cellularity is due to an endothelial proliferation and to the presence between the capillaries of large cells with a foamy cytoplasm and a nucleus very similar to that of the endothelial cells. There is a very marked thickening of the capillary basement membrane and the basement membrane of the parietal layer of Bowman's capsule. The thickening, often associated with multiplication of layers, is frequently crescentric. The crescent is usually opposite to the vascular pole but sometimes on one side. Few glomeruli show advanced hyalinisation or fibrosis and shrinkage. There is no epithelial proliferation and no formation of epithelial demilunes. Only very occasionally a patch of glomerular necrosis is seen. The tubuli show in their epithelial lining little difference between the various segments. The epithelium is cubical or low columnar and shows marked hydropic degeneration. Here and there necrosis of tubular epithelium is seen. The majority of tubuli have dilated lumina, a minority are atrophic. The tubuli are separated by a very considerably widened interstitium. The latter is markedly cellular with predominance of large mononuclear cells and small round cells, a few fibroblasts and tissue mast cells. In this interstitium an abundance of birefringent crystalline material is seen in the cortex as well as in the medulla. Many crystals are also present in the fibrous capsule of the kidney. Occasionally a few minute birefringent crystals are seen in the mesangium of a glomerulus. The arterial changes are focal, but the majority of interlobular arteries and proximal segments of afferent arterioles show media hypertrophy or duplication of internal elastic membrane and subendothelial intima proliferation.

Spleen : The Malpigian corpuscles are rather small, the venous sinuses for the most part empty, the cords of Billroth very cellular. The most striking finding, particularly in azan stained sections, is the presence of nests or cords of large cells with a diameter of 18–25μ, a small dark, pale or absent nucleus and a foamy, sky-blue cytoplasm (Fig. 1). These nests and cords are mainly in the Billroth's cords of the red pulp, but also around the follicular arteries and within the trabeculae. In some of the latter the reticulin and collagenous fibres form a network with wide meshes which are filled with these foamy cells. There is an abundance of crystalline birefringent material in the red pulp, around the follicular arteries and in the trabeculae. In many places there is such a heavy deposit of crystals that it is impossible to define their localisation. However, not infrequently the localisation

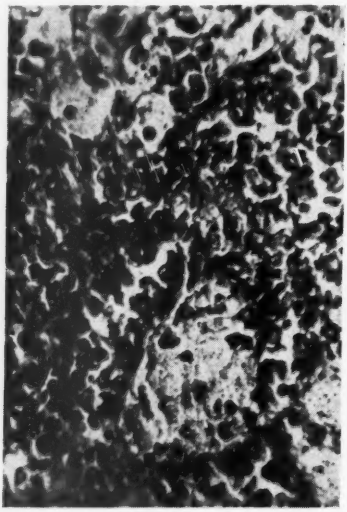


Fig. 1. Case 4. Spleen. Azan. ×625, linear reduction $\frac{1}{2}$.

in the reticulum cells of Billroth's cords is clear while only exceptionally a tiny crystal is seen in a littoral cell of a venous sinus.

Liver : The lobular structure and the trabecular arrangement of liver cells are well preserved. The capillaries contain few red blood cells and the Disse spaces are markedly dilated. The Kupffer cells are greatly swollen, 20–30μ in diameter. and they often form nests between liver cells which completely obliterate the capillaries (Fig. 2). Both, cells clearly identifiable as Kupffer cells and cells forming sharply demarcated nests, have the same foamy cytoplasm, showing pale blue in azan stain. In the largest of these cells no nucleus is present, in many there are granules of nuclear debris, while those without necrobiotic changes have small dark nuclei. The majority of such nests of foamy cells are intralobular but occasionally small nests may also be seen in periportal spaces, either in the adventitia of portal veins or closely attached to a bile duct. Abundant birefringent crystalline

material is seen in the liver in sections stained with basic fuchsin and its distribution shows a striking resemblance to the distribution of foam cells as seen in azan-stained sections where the crystals had been dissolved. The liver cells show moderate fatty changes and in none are crystals seen. In some liver cells a severe hydropic swelling of nuclei is present.

Phosphatase is not demonstrable in any of the available organs, as only formalin-fixed material is at our disposal.

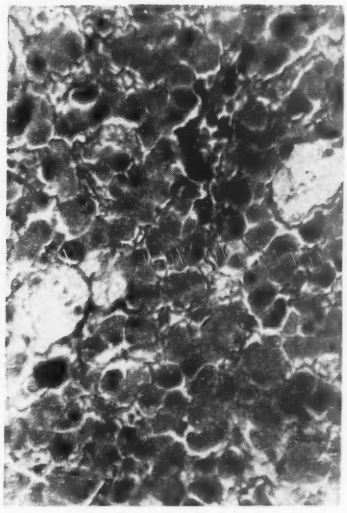


Fig. 2. Case 4. Liver. Azan. ×380, linear reduction ½.

Case 7

R.O., a boy aged 2½ years. Autopsy performed by Dr. O. C. Lloyd. Cystine crystals in a bone-marrow film from this case are seen in Fig. 3.

The histological examination gave the following findings :

Kidneys : Some of the glomeruli are normal in appearance, but the majority show a moderately increased cellularity. This is due to endothelial cells and large cells in the mesangium, which have a foamy cytoplasm and a nucleus resembling that of endothelial cells. Occasionally a cell with a segmented nucleus is seen within a tuft, apparently a polymorphonuclear leucocyte. Some glomeruli have very few patent capillaries ; these are distended and usually situated in the periphery of the tuft. Other glomeruli show no patent capillaries at all. Occasionally a patch is seen within a glomerulus which stains pale yellow-brown with van Gieson and within which only a few tiny, pale nuclear remnants are discernible. There is nowhere epithelial proliferation. The basement membranes of Bowman's capsule are slightly thickened and thickening of capillary basement membranes is also encountered. Very occasionally a narrow band which stains red with van Gieson

is seen within a glomerulus. While the majority of glomeruli show only very mild changes an occasional completely hyalinised glomerulus is also found. The epithelium of proximal convoluted tubuli is swollen, their cytoplasm finely granular. Some of the epithelial cells are disintegrating with granular material in the lumen of the tubuli but most cells, though swollen, have a clearly demonstrable brush border. No changes are seen in the distal segments of the tubuli. There is a slight oedema of interstitial tissue in the cortex, but not in the medulla. It is difficult to decide how much of the separation of tubuli is due to oedema and how much to shrinkage by alcohol fixation. There are, however, places where faintly eosinophilic material is seen between the tubuli and the presence of oedema becomes quite definite by silver impregnation. The argentofilic fibres are much more conspicuous than normal and there is swelling and splitting with a brownish material between separated fibrils. There are no vascular changes. No crystals are seen.

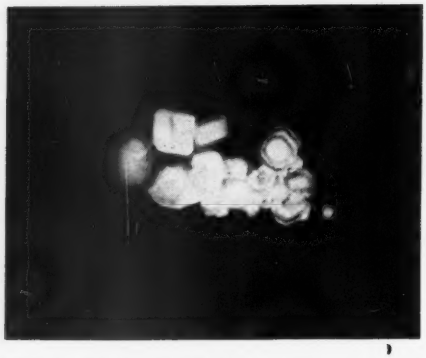


Fig. 3. Case 7. Crystals in bone-marrow smear. Crossed Nicols. ×370.

Liver : There is a large number of white blood cells in the capillaries. The Disse spaces are distended and the Kupffer cells are swollen. The moderately swollen Kupffer cells are elongated and their cytoplasm stains dark purplish-blue with azan. The severely swollen Kupffer cells are ovoid or pyramidal in shape, their cytoplasm is pale blue and foamy. There is a moderate cellular infiltration of periportal spaces but only exceptionally a foam cell is seen in such a space. Only few birefringent granules are seen ; none of these resemble cystine crystals.

Spleen : The Malpighian corpuscles are large and show large germinal centres. The red pulp is highly cellular, its venous sinuses are, except for the perifollicular sinus, narrow. The Billroth's cords are broad and densely cellular. Only very few reticulum cells of the cords show with azan stain a pale blue foamy cytoplasm and a small nucleus like the cells in typical cases of cystinosis. The trabeculae and blood vessels are normal. No crystalline material is seen.

Heart : Oedema of interstitial tissue.

Thymus : Normal, no atrophy or fatty metamorphosis.

Pancreas : Nil abnormal.

Eye : Only the lens with ciliary body and a fragment of pars ciliaris retinae received. All these structures are normal.

Suprarenal : Normal.

Bone-Marrow (Specimen 1) : The cellular composition is normal. There are numerous tiny granules of doubly refractile material. Such granules are not uncommonly seen in normal marrow though usually in smaller numbers. They do not resemble cystine and such crystals as have been found in the bone-marrow films intra vitam were not found in the post mortem material in spite of thorough search.

Bone-Marrow (Specimen 2) : This is fatty with islands of active marrow. In the latter there are numerous ovoid or elongated cells up to 30μ in diameter with a small eccentric nucleus and a foamy cytoplasm. No alkaline phosphatase is demonstrable in any of the alcohol-fixed organs by the Gomori II method.

Case 9

M.B., a girl aged 9 years. The post mortem examination was carried out approximately 40 minutes after death by H.S.B. Body of a markedly dwarfed female child. Length 40 ins., arm span 39 ins., head circumference 20$\frac{3}{4}$ ins. Spina iliaca anterior superior to malleolus externus 18$\frac{3}{4}$ ins., spina iliaca anterior superior to lower end of patella 10$\frac{1}{2}$ ins., acromion to tip of middle finger 17$\frac{1}{2}$ ins., acromion to olecranon 8$\frac{1}{2}$ ins. The proximal third of both femora was bowed outward, more on the right side, and knock-knees were present. There was no widening of the distal epiphyses of the fore-arm and no rachitic rosary. Complexion pale, with a definite yellowish tinge. There were blood crusts in both nostrils, some haemorrhages and superficial ulceration at the lips and at the left angle of the mouth. There were numerous pinhead to millet-sized petechiae over the front of the chest and in the epigastric region, less numerous on the rest of the abdominal wall and on the back. There was also an occasional ecchymosis in the skin of the face and left leg and numerous pricks for intravenous injections were surrounded by ecchymoses. There was a swelling between the inner and middle thirds of the left clavicle apparently due to callus. There was moderate oedema of the legs, especially the right, and in the face. The upper lids showed a slight suffusion and oedema and the eye-balls were rather firm on palpation. The hair was of a pale, Cotswold stone colour and up to 10 ins. in length. There was no abnormal growth of hairs anywhere on the body. The panniculus adiposus of the anterior abdominal wall was 3 mm. in thickness and the fat was of a saffron-yellow colour. The muscles were normal in colour.

Neck and Thorax

Tongue : Yellowish-white coated. Several superficial irregular ulcers with granular, bright red floors on the dorsum of the tongue. A few similar ulcers were present in the mucous membrane of the **cheeks** and **lips**.

Submaxillary Glands : 20×12×6 mm. in size, pale yellowish-white in colour.

Right Tonsil : 16×6×5 mm. in size ; its surface was pinkish-grey and showed shallow crypts. The cut surface was yellowish-pink and no pus was seen.

Left Tonsil : 10×8×5 mm. It was yellowish-pink on the surface and showed two deep crypts, one with yellowish-red pus. The cut surface was similar to that of the right tonsil and no deep abscesses were seen.

Right Upper Parathyroid : 5×3×2 mm. in size.

Right Lower Parathyroid : 4×2×2 mm. in size.

Left Lower Parathyroid : 4×2.5×1.5 mm. in size.

Left Upper Parathyroid : Not definitely identified.

Thyroid : Left lobe 25×8×3 mm., right lobe 30×9×5 mm. The thyroid was rather small and pale yellowish-grey in colour.

Cervical Lymph Nodes : Not enlarged.

Thymus : Grossly atrophic.

Pharynx : A few pinhead-sized submucous petechiae.

Trachea : Normal, its mucous membrane pale.

Large Bronchi : No exudate.

Pleurae : Normal, except for a few pinhead to millet-sized subpleural haemorrhages over each lung.

Lungs : Pale pinkish-grey in colour, moderately well aerated. There was no increased amount of fluid on the cut surfaces, no pus in the bronchi and no areas of consolidation.

There was a fair amount of **pericardial** fat, which was saffron-yellow in colour. There were numerous pinpoint to pinhead-sized subepicardial haemorrhages present.

Heart : Somewhat larger than the closed fist, 7.7×6.5×2–3 cm. in size ; weight 96.5 g. The right ventricle was flabby, the left ventricle well contracted. The thickness of the wall of the left ventricle was 13–14 mm., that of the right ventricle 2–3 mm. Valves and septa normal. The intima of **aorta** showed a diffuse pale yellowish discolouration with soft, slightly elevated, ill-demarcated, orange-yellow patches in the ascending aorta just above the semilunar cusps and in the descending aorta.

Tracheo-bronchial Lymph Nodes : Not enlarged. Those at the bifurcation lentil-sized and blackish in colour.

Abdomen : About 2 pints of slightly turbid, reddish fluid in the abdominal cavity.

Stomach : Empty, mucous membrane normal. There was an occasional subserous haemorrhage over the stomach.

Duodenum : Contained bile-stained slime ; mucous membrane normal.

Small Intestine : Several pinhead-sized subserous haemorrhages. There was a single agonal intussusception in the **jejunum**. The mucous membrane of the small intestine showed no pathological changes, except for the distal part of the **ileum** where several submucous haemorrhages were present. There were no ulcers and no definite scarring. In the whole colon were numerous millet to lentil-sized, dark red submucous nodules with superficial ulceration, to which thin grey pseudo-membranes and faecal masses were adherent. Corresponding to the taeniae there were longitudinal ridges markedly elevated and of the same appearance as the previously mentioned nodules.

Mesenteric Lymph Nodes : Lentil-sized. In the ileocaecal region there was one pea-sized node and this was surrounded by a group of lentil-sized nodes which were firmer in consistency than the others and all these showed on the cut surfaces yellowish areas of caseous necrosis.

Liver : 539g. in weight, 22×13×10 cm. in size. It was pale fawn in colour and the capsule showed scattered, fine areas of milky-white thickening. The cut surface was somewhat darker, brownish-yellow in colour and the lobular structure was very indistinct. The consistency of the liver was normal. When the

cut surface was examined with a magnifying glass it appeared powdered with minute white granules.

Gall Bladder : Distended with reddish-brown, rather thin bile.

Extra-hepatic Bile Ducts : Patent.

Spleen : $10 \times 4 \times 2\frac{1}{2}$ cm. in size, 63.5 g. in weight. Pale salmon-red in colour with patches of white capsular thickening in the cranial third. The cut surface was greyish-red in colour and showed conspicuous trabeculae, especially in the sub-capsular area. The Malpighian follicles were not discernible and all through the pulp were peculiar, pinhead-sized cystic spaces. When the cut surface was examined with a magnifying glass it appeared powdered with minute white granules.

Kidneys—Left : $55 \times 22 \times 20$ mm. in size, 20.5 g. in weight (normal 83 g.). The capsule was adherent and only with difficulty detached ; the colour was pale yellowish-pink, the surface finely granular with a few pinpoint-sized haemorrhagic spots. On the cut surface practically no normal markings were discernible and there was pale pink tissue with pinpoint-sized, reddish spots and narrow greyish strands.

Right : $55 \times 25 \times 20$ mm. in size, 21.75 g. in weight, otherwise identical with the left kidney. When the cut surfaces of the kidneys were examined with a magnifying glass they appeared powdered with minute white granules.

Urinary Bladder : Showed several pinhead-sized submucous haemorrhages mainly in the region of the trigonum.

Uterus : $3 \times 1 \times 0.6$ cm. in size.

Ovaries : Right : $15 \times 5 \times 2$ cm., Left : $15 \times 6 \times 3$ cm. in size.

Pancreas : Normal in size and consistency, 18 g. in weight. The cut surface was pale yellow, lobular structure distinct. There was an occasional enlarged para-pancreatic lymph node, $9 \times 7 \times 3$mm. in size.

Suprarenals—Left : $4.5 \times 2.5 \times 0.7$ cm. in size, 7.3 g. in weight, reddish in colour. On the cut surface only small patches of yellow lipids were seen in the cortex, otherwise the cortex was dark blackish-red in colour and surrounded a rather bright orange-yellow medulla. **Right** : $4.5 \times 3 \times 0.5$ cm. in size, $10\frac{1}{4}$ g. in weight and had on the whole cut surface the same appearance as the left suprarenal.

Cranium : The vault of the **skull** was rather thin, most of it was 1–2 mm. in thickness, with some transparent areas, but with no flexible areas.

Dura Mater and its venous sinuses : Normal. There was an increased amount of fluid in the subarachnoid space over the whole brain and the fluid was definitely, although slightly turbid in the parieto-occipital region. A pure culture of a mucoid E. coli was obtained from the **leptomeninges**.

Brain : Oedematous.

Spinal Cord and its meninges : normal.

Middle Ears : Thin, reddish fluid in both.

Para-Nasal Sinuses : No pus.

Bones—Right Femur : Showed a transverse fracture about 5 cm. below its neck ; distal to it there was a thickening of the shaft, due to callus. At the lower end of the femur a plate of bluish transparent cartilage, 7 mm. in thickness, separated the epiphysial ossification centre from the diaphysis. This cartilaginous plate was quite irregularly outlined on the diaphyseal side and showed a patchy deposition of lime salts, but no continuous provisional calcification zone was present (Fig. 4). The bone-marrow was fatty in the metaphysis, pale brick-red in the diaphysis.

Costo-chondral Junctions : The provisional calcification zone was irregular and interrupted in the marginal parts. The proliferation zone was slightly widened. The bones were rather easily sawn through and the ribs also easily cut through with a knife. Bone-marrow was pale greyish-pink.

Left Clavicle : Showed a cherry-sized callus at the junction of the proximal and middle thirds.

Histological Examination

Tonsils : There is homogenous eosinophilic material in the crypts with some deposit of lime salts. There is an abundance of crystalline material in the subepithelial connective tissue and in the reticulum between the lymph follicles, almost none within the follicles. Where crystalline deposition is seen, especially in the subepithelial tissue, there is also atrophy of lymphoid tissue.

Fig. 4. Case 9. Lower end of femur. Partial decalcification. H.E. ×8, linear reduction ½.

Submaxillary Gland : There is an abundance of crystalline material in the interstitial tissue, otherwise the structure is normal and no crystals are seen in the secretory or duct cells.

Parotid Gland : There are pus cells in the ducts and in a few places there is purulent infiltration of the gland with destruction of secretory cells. There are numerous crystals in the interstitial tissue except where suppuration has taken place.

Tongue : There is ulceration of the mucosa, submucosa and superficial muscle layers. The floor of the ulcer is covered by pus cells, fibrin and nuclear debris. There is marked polymorphonuclear infiltration around and below the ulcer. No crystals are seen in the sectioned area.

Thyroid : There is a moderate broadening of the interstitial tissue and a fair number of crystals are seen in the interstitial tissue : none in the epithelial lining of vesicles. The vesicles are distended with pale homogenous, occasionally vacuolated colloid.

Parathyroids : Two examined histologically ; the size of one in the section is 1.9×1.35 mm., the other 2.3×1.95 mm. The structure is compact and there are a

few fat cells. Approximately three-quarters of the cells are cloudy chief cells and one-quarter water-clear cells ; no eosinophil cells. Some nuclei are hyperchromatic; a few multinuclear cells with water-clear cytoplasm are seen. A few crystals are present in the perivascular tissue.

Lymph Nodes (upper deep cervical, peri-pancreatic, mesenteric) : All lymph nodes except the mesenteric show identical changes. There is marked sinus catarrh, abundance of crystalline material is present in the reticulum of medullary cords between the lymph sinuses. Only in a few places do littoral cells contain crystals, namely, either cells lining sinuses or cells which have been shed off into the lumen of lymph sinuses. In a few places where crystalline material is most abundant free crystals are also seen in the lumen of the lymph sinuses. The difference between the inter-sinusal reticulum and the littoral cells is particularly striking in the periphery of the nodes where the marginal sinus is practically free of crystals and the follicles contain only a few crystals within the germinal centres, while an abundance of crystals is seen in the medullary cords.

A Mesenteric Lymph Node : Shows complete uniform caseous necrosis with narrow strands of lymphocytes in the periphery and a broad dense fibrous capsule. No acid-alcohol fast bacilli are found. There is no crystalline material in this lymph node.

Lungs : There are a few areas of collapse. A moderate round cell infiltration of the interstitial tissue including the interalveolar septa is seen in a few places. Alveolar macrophages are present in many air vesicles. Only after prolonged search can a very occasional birefringent crystal be seen in an interalveolar septum.

Heart : There are no significant pathological changes and no crystals are seen.

Aorta : The structure is normal. A few crystalline deposits are seen in the adventitia.

Thymus : Extreme atrophy of lymphoid component. Hassall's corpuscles are numerous and large.

Liver : There are marked fatty changes. The Disse spaces are very considerably distended and the capillaries are compressed, occupying less than half of the space between the liver cell trabeculae. The Kupffer cells are everywhere considerably swollen, some elongated, some ovoid and some with pseudopodial cytoplasmatic processes ; the cells range up to $20 \times 15\mu$ in size. Their cytoplasm is foamy and shows in azan stain fine bluish lines separated by clear areas (Fig. 5). Crystalline material is seen in Kupffer cells, in the connective tissue of periportal spaces and there is occasionally a free crystal found in a Disse space.

Spleen : The capsule is normal in thickness. The Malpighian corpuscles are rather small and show no germinal centres. The red pulp is cellular ; its venous sinuses variable in width, some distended, others collapsed. In the cords of Billroth many large cells, 12–25μ in diameter, are seen which have a pale blue, almost colourless cytoplasm in haematoxylin-eosin stained preparations, while with azan-staining the cytoplasm appears pale greyish-blue, foamy or finely granular (Fig. 6). The nuclei of these cells are spherical, ovoid or kidney-shaped, with a distinct nuclear membrane and occasionally with a single small nucleolus. In many of these cells the nucleus is very pale. Such nuclei show a nuclear membrane and granular chromatin condensations within a colourless material. Other nuclei are small pyknotic or show karyorhexis. Occasionally granules which take nuclear stain are seen within the cytoplasm. Such cells usually show no nucleus. The cells with necrobiotic nuclear changes are as a rule larger than those with normal nuclei.

The littoral cells of the venous sinuses are often markedly swollen, but do not show the cytoplasm of the above described reticulum cells of the Billroth's cords. The trabeculae and trabecular veins are normal. Several of the trabecular arteries and follicular arteries show fragmentation and duplication of the elastic membrane and subendothelial intima proliferation. Birefringent prisms and hexagonal plates are numerous. They are seen mainly in clumps which in size and shape correspond to the above described cells of Billroth's cords. Often a nucleus is discernible within a cluster of crystals or a group of 2–4 nuclei is surrounded by closely packed crystals. Only occasionally do very small single crystals appear to be lying between the cells. In the Malpighian corpuscles crystals are considerably less numerous than in the red pulp. Where their intracellular situation is recognizable they are in reticulum cells between lymphocytes and in the adventitia of follicular arteries, but never in

Fig. 5. Case 9. Liver. Azan. ×625, linear reduction ½.

lymphocytes. Frequently crystals form rows along the borders of trabeculae while within the trabeculae there are only few crystals. Where a trabecula contains an artery there may be more crystals around the artery but no such localisation is seen around trabecular veins. The littoral cells of venous sinuses are with few exceptions free from crystals.

Kidneys : Both kidneys show the same changes. There is a complete disorganisation of the renal parenchyma with an enormous, but irregularly distributed increase in the amount of connective tissue in the medulla as well as in the cortex. This interstitial tissue is in some areas very cellular with lymphocytes and large mononuclear cells (Fig. 7) while in other areas it is fibrous and shows a low cellularity. Scattered throughout the interstitium, but more in the medulla and in the submucosa of the renal pelvis, are numerous tissue mast cells (Fig. 8). Some

glomeruli show only mild changes, others are completely transformed into a small fibrous ball. The majority of the glomeruli are bloodless and contain no patent capillaries. In many there is a patchy necrosis of the tuft (Fig. 9). Such necrotic anuclear areas stain brown or yellow with van Gieson stain and are finely granular. Red-staining homogenous material only appears in the glomeruli with more advanced changes and is always first seen in the periphery. In such glomeruli there is always marked hyaline-thickening of the basement membrane of the parietal layer of Bowman's capsule ; this is particularly clear when periodic acid-Schiff's reagent stain is carried out. Whenever fibrotic changes occur in the glomeruli it is obvious that glomerular fibrosis was preceded by periglomerular fibrosis. In a few glomeruli there is an increase in cellularity, mainly due to proliferation of capillary endothelium and cells in the mesangium, but occasionally also to proliferation of the visceral

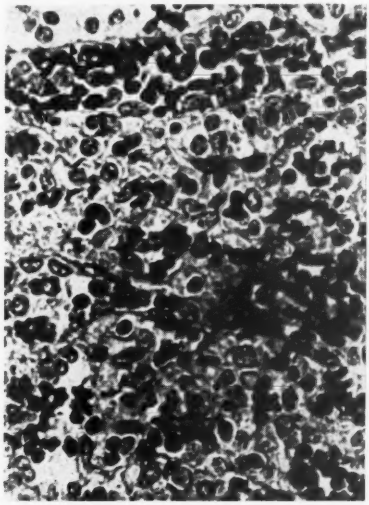


Fig. 6. Case 9. Spleen. Azan. ×625, linear reduction $\frac{1}{2}$.

layer of Bowman's capsule. Whenever this is present the nuclei of the capsular epithelium are dark, and occasionally binuclear epithelial cells may be seen (Fig. 10). Rarely crescent formation and/or adhesion between tuft and capsular epithelium are noted. All tubuli show severe changes of their epithelial lining which is mostly modified into a cubical epithelium, so that except for the spacial relationship to glomeruli it is impossible to differentiate the various segments of the tubuli. In some areas tubuli have entirely disappeared and are replaced by granulation tissue ; in other areas they are dilated, contain granular and hyaline casts or show transformation into cystic spaces with colloid-like material, like those seen in chronic pyelonephritis. When stained with Weigert's fibrin stain fibrils staining like fibrin are occasionally seen in and around glomeruli (Fig. 11). The interlobular arteries are normal, but many of the afferent arterioles show media hypertrophy, intima proliferation and, in their proximal segments, elastic duplication. Crystals are

comparatively scanty, but are more numerous in the medulla than in the cortex. Occasionally a few crystals are seen in the mesangium of a glomerulus (Fig. 12) and rarely in the space of Bowman's capsule. Very occasionally crystals may be seen in the lumen of a tubulus, apparently in the proximal convoluted portion, and may then fill the whole lumen forming a minute calculus (Fig. 13). Crystals were never seen in the epithelial cells of the tubuli.

Fig. 7. Case 9. Kidney, round cell infiltration of medulla. Van Gieson. ×150, linear reduction app. $\frac{1}{2}$.

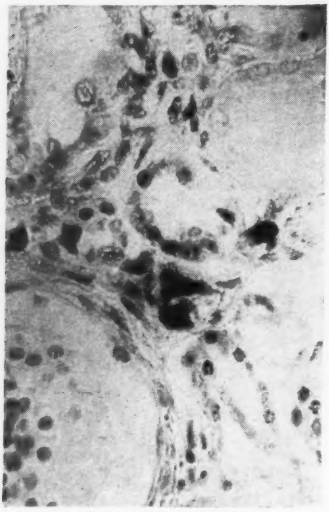


Fig. 8. Case 9. Kidney showing tissue mast cells. Alcoholic thionin. ×250, linear reduction 6/10.

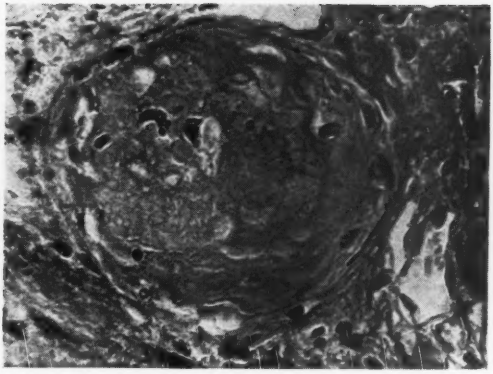


Fig. 9. Case 9. Kidney. Van Gieson. ×625, linear reduction ½.

Fig. 10. Case 9. Kidney. Azan. ×625, linear reduction ½.

Fig. 11. Case 9. Kidney. Weigert's fibrin stain. ×192, linear reduction ½.

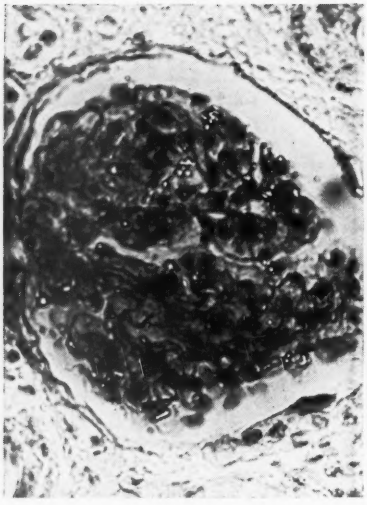


Fig. 12. Case 9. Kidney. Alcohol fixation. Basic fuchsin. ×625, linear reduction ½.

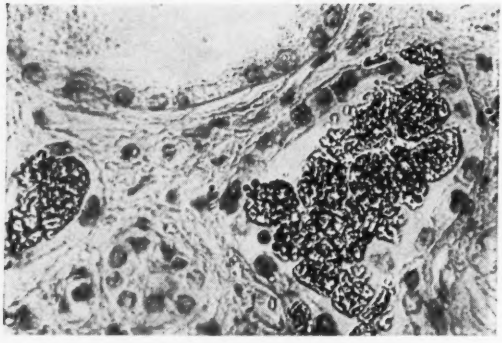


Fig. 13. Case 9. Kidney. Two cystine-microliths in proximal convoluted tubules. Alcohol fixation. Basic fuchsin. ×625, linear reduction ½.

Pancreas : There is a microcystic dilatation of intralobular ducts and acini. These small cysts are lined by a modified cubical epithelium and contain eosinophilic material. The islands are normal. Crystalline material is present in the interlobular connective tissue.

Stomach : There is almost complete absence of parietal cells of the fundus, while chief cells are normal. Crystals are seen in the lamina propria and in the submucosa. There is a moderate round cell infiltration of the lamina propria.

Small Intestine : Paneth's cells are almost completely absent. Crystals are present in the lamina propria, within the lymph follicles, more rarely in the submucosa, very seldom between the fibres of the muscular coat and never in the subserosa.

Large Intestine : Crystalline deposits in the lamina propria and submucosa, otherwise no pathological changes.

Pituitary : A fair amount of crystalline material in the interstitial tissue, otherwise nil abnormal.

Brain : Heavy infiltration of leptomeninges with lymphocytes, large mononuclear and polymorphonuclear leucocytes. Oedema of brain. The choroid plexus is oedematous and its stroma contains large mononuclear cells with foamy cytoplasm. No crystals seen.

Eye : Fixed in mercuric chloride-formalin. The mercuric chloride formed with the crystals blackish, amorphous material which is abundant in the sclerae, conjunctivae, iris and cornea.

Skeletal System

Ossification centre at lower epiphysis of Femur : The bone trabeculae are thin and separated by broad bands of purely fatty marrow. All trabeculae show broad osteoid seams. Osteoclasts and Howship's lacunae are scanty except at the junction with the cartilage, where the bone-marrow is in a few places fibrous and the number of osteoclasts somewhat increased.

Distal ossification zone of Femur : There is a wide proliferation zone of the cartilage, but only in a few places cell columns are arranged in the long axis of the bone. In many places they form various angles with this axis. The whole zone is highly vascularised and a broad cartilage marrow canal is seen at the junction of resting cartilage with the proliferation zone. Connective tissue penetrates the cartilage with the blood vessels and within this connective tissue trabeculae of osteoid with a little calcified bone are seen. By the proliferation of cartilage marrow canals and by invasion of the cartilage by vascular connective tissue from the shaft, the epiphysial cartilage is disrupted and in some places islands of cartilage are surrounded by connective tissue with or without osteoid or bone trabeculae (Fig. 4). There is no continuous preparatory calcification zone, but patches of calcium deposits are seen in the cartilage. The bone trabeculae of the primary spongiosa are quite irregular and some run perpendicular to the axis of the shaft. The bone trabeculae have broad osteoid seams ; their osteoblastic lining consists of flat cells. Osteoclasts and Howship's lacunae are scanty, though there is evidence of dissecting bone resorption and formation of compound bone trabeculae. The bone has mostly the structure of Haversian bone.

Proximal end of Femur : The changes are less severe than at the distal end. The osteoid seams are broad. The bone-marrow is more cellular. Only where the marrow tissue penetrates the cartilage is it fibroblastic.

Sternum : Bone trabeculae and trabeculae of calcified cartilage matrix are surrounded by broad osteoid seams. The bone-marrow is cellular and haematopoietic, with scanty osteoclasts.

Vertebrae : The provisional calcification zone is interrupted in a few places. The primary trabeculae are irregular in their course, oblique or perpendicular to the long axis. The osteoid seams are 40–80μ in width, the bone-marrow is cellular. There is very little bone resorption. The bone structure is that of Haversian bone.

Rib : The columnar zone of the cartilage proliferation is markedly increased in width. The columns in the centre of the rib are parallel to the long axis, while in the periphery their direction is more oblique. The provisional calcification zone is defective and shows only patches of lime salt deposits. In the centre the calcification zone and about one-third of the columnar zone are penetrated by a mushroom-shaped extension of very vascular fibrous marrow tissue (Fig. 14). The cartilage marrow canals at the junction of the resting and proliferating cartilage are vascular and cellular. Except for the part penetrating the cartilage the marrow is haematopoietic with a moderate number of fat cells. In the fibrous as well as in the haematopoietic part of the marrow there are numerous large cells with a foamy cytoplasm which stains pale blue with azan. Neither in the cellular nor in the fibrous area is the number of osteoclasts increased. The zone of primary bone trabeculae is very short and followed by secondary trabeculae of approximately normal width. All trabeculae show marked but not very wide osteoid seams.

Bone-Marrow of Femur : The marrow is cellular, shows some increase in the number of erythroblasts and a prevalence of immature myeloid cells. There are numerous crystals, which are either distinctly intracellular or form clusters in the shape of an enormously large cell (Figs. 15a and b). Within such a cluster a nucleus

Fig. 14.

Case 9. Rib. Partial decalcification with Müller's fluid. H.E. ×48, linear reduction $\frac{1}{2}$.

may be seen or a few nuclei surrounded by aggregates of crystals, indicating the intracellular origin of the crystals.

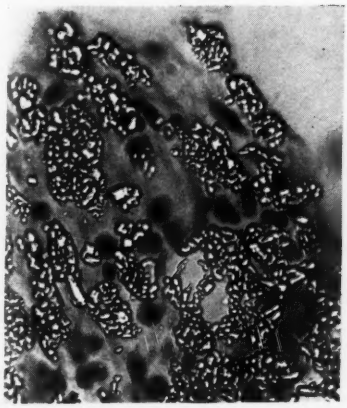


Fig. 15a. Case 9. Bone-marrow. Alcohol fixation. Basic fuchsin. ×625, linear reduction ½.

Fig. 15b. Case 9. Bone-marrow film. Crossed Nicols. ×800.

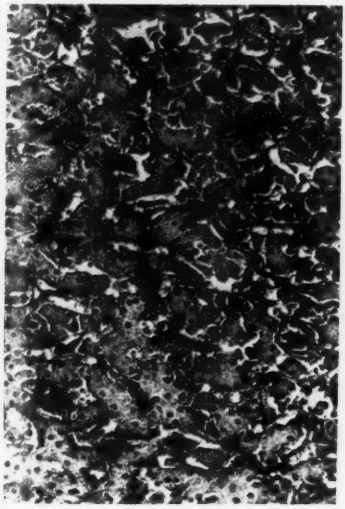


Fig. 16. Normal liver. Phosphatase. ×120, linear reduction 2/3.

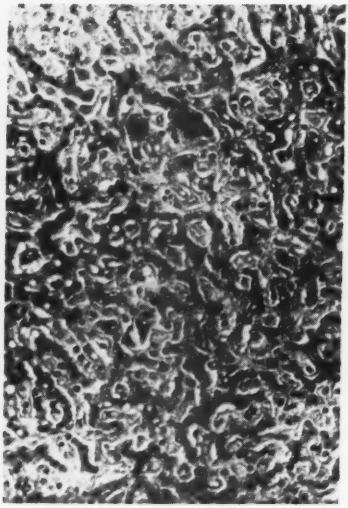


Fig. 17. Case 9. Liver. Phosphatase. ×120, linear reduction 2/3.

Histochemical demonstration of phosphatase was carried out by Gomori's second method. Tissues of a 9 year old child with perityphlitic abscess, on whom the post mortem examination was made 16 hours after death, were used as controls. There is no phosphatase in the kidneys of M.B. and much less than in the control in liver, spleen and bone-marrow (Figs. 16–21). There is, however, no difference in the phosphatase reaction of the small intestine.

Fig. 18.
Normal bone-marrow. Phosphatase. ×625, linear reduction 6/10.

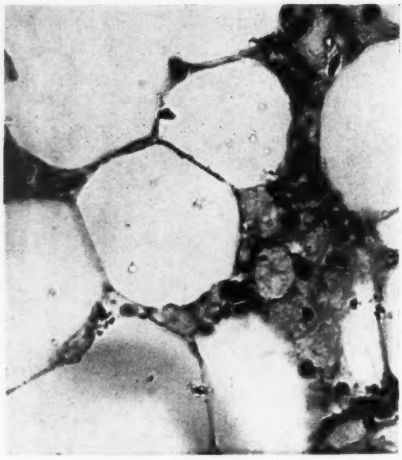


Fig. 19.
Case 9. Bone-marrow. Phosphatase. ×480, linear reduction 6/10.

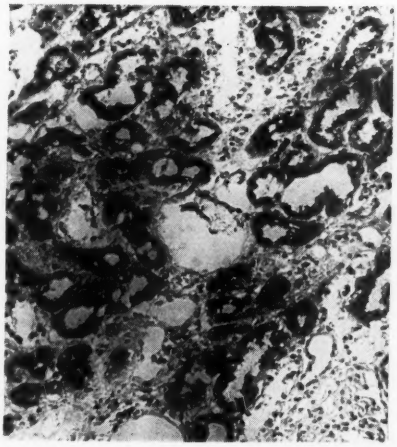


Fig. 20.
Normal kidney. Phosphatase.
×120, linear reduction 6/10.

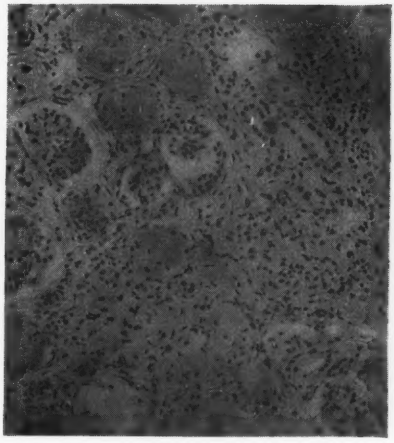


Fig. 21.
Case 9. Kidney. Phosphatase.
×120, linear reduction 6/10.

Case 11

D.L., a boy aged 15. Autopsy performed approximately 48 hours after death by Dr. J. Emery. The external appearance showed a wasted, contorted child. No external anatomical abnormalities. Oedema present at scrotum, back of hands and over the sacrum. There was a fine, papular rash over almost the whole of the body, most marked over the back of the shoulders and upper abdomen. Over the abdomen the papules were pale, whereas over the shoulders and back they were deep red and at first sight appeared to be almost purpuric.

Abdominal Cavity : Contained about 100 ml. of straw-coloured fluid, but there were no adhesions.

Pericardial sac : Contained about 20 ml. of straw-coloured fluid.

Pleural Cavities : Each contained 500–1,000 ml. of fluid, more on the right side than on the left. The lungs were partially collapsed.

Respiratory Tract : Nose, larynx, trachea and bronchi contained fragments of blackish-green fluid similar to that present in the stomach and apparently due to terminal inhalation. No pus or anatomical deformities in the respiratory tract. Tonsils appeared normal, small.

Lungs : Some small areas of apparent consolidation in the upper lobes. The lower lobes only appeared to be collapsed. No brown induration or abnormal congestion. Primary tuberculous complex not identified. Glands at the hilum deep brownish-red. No caseation seen, glands not unusually enlarged.

Heart : Grossly enlarged, 240 g. in weight. This was possibly due to the relative collapse of the lung. The left side of the heart appeared considerably more prominent than the right. On cutting, valves and cavities of the heart appeared normal ; increased thickness of the ventricular walls. Muscle appeared normal and the coronary vessels arose normally and showed no naked eye abnormalities. Foramen ovale obliterated by a pale membrane. Ductus arteriosus closed. Great vessels showed no gross abnormalities.

Alimentary Tract : Tongue furred ; papillae at back of tongue prominent. Oesophagus normal. Mucosa of stomach was thrown in fairly high folds, some of which were bright red, particularly at the fundus. Near the pylorus the mucosa appeared thinner and not congested, but the stomach contained about 300 ml. of thin, green fluid containing a large number of blackish shreds similar to those seen in the trachea ; these appeared to be altered blood. The pylorus was normal. Intestinal tract showed no gross abnormality throughout its length. Peyer's patches not unduly prominent. No abnormality seen in the mucosa. Large bowel contracted, normal. Rectum and anus normal.

Liver : 829 g. in weight, externally a little pale, on section a little oedematous, otherwise no abnormality seen.

Gall Bladder : Normal amount of bile, showing no abnormality.

Spleen : 81 g. in weight, firm and rather pale. No gross abnormalities.

Kidneys : Relatively small, both 70 g. in weight. Capsules not unusually adherent ; surface slightly irregular. The renal arteries showed no abnormality on the left side ; on the right side there was a large aberrant vessel running across the ureter to the right of the kidney but causing no structural change to the kidney pelvis. On section less differentiation than usual ; cortex appeared to be slightly thin.

Urinary Bladder : Normal, containing about 200 ml. of normal urine.

Testis : Descended. **Prostate** : Normal, small. **Penis** : Normal, circumcision.

Adrenals : Each 10.5 g. in weight, thin and yellow, within normal limits.

Pancreas : Very firm, normal.

Thymus : Considerably less wasted than expected.

Thyroid : Appeared normal, 6 g. in weight.

Parathyroids : 2 identified at the lower poles of the thyroid, each approximately $3 \times 2 \times 2$ mm. in size and 1.466 g. in weight.

Brain : Externally no gross abnormality apart from some slight congestion. On gross section no abnormality seen apart from a large, pale, soft area approximately $10 \times 10 \times 6$ mm., lying in the choroid plexus of the right lateral ventricle.

Pituitary : Normal.

Eyes : Both removed. There was distension of the membrane surrounding the optic nerve immediately behind the eyeball.

Skeletal System : Gross deformities were present and are well illustrated in the X-ray pictures (see Part 7).

Connective Tissue : Slight general oedema.

Fat : No abnormality throughout the body.

Lymph Glands : No noticeable enlargement ; probably within normal limits.

Histological Examination

Kidney : There is a complete disorganisation of the structure. Many glomeruli are transformed into small, almost anuclear balls which stain pink with haematoxylin and eosin, blue with azan. The material which replaces the glomeruli is frequently granular, and the periphery of the sphere often stains deeper than the centre. In many such glomeruli there is a pericapsular shell of several layers of hyaline bands or collagenous fibres. Other glomeruli are normal in size or enlarged. These are either bloodless or show a few distended capillaries. Their cellularity is mainly due to endothelial proliferation, but partly also to the presence of large mononuclear cells with a foamy cytoplasm which stains sky-blue with azocarmine. There is also a proliferation of capsular epithelium and adhesions between tufts and parietal layer of Bowman's capsule. Some of the comparatively cellular glomeruli show patchy areas of necrosis which stain pale pink with haematoxylin-eosin, pale blue with azocarmine and brownish-yellow with van Gieson's stain. The tubuli are frequently dilated and all lined by a modified flat, cubical, rarely low columnar epithelium. A classification of tubular segments is impossible, though in some the epithelial lining has a clear colourless cytoplasm while in others there are fuchsinophil granules in the cytoplasm. In some of the most dilated tubuli there is a proliferation of epithelium with formation of spur-like projections into the lumen. In many places the tubuli are atrophic, in others they have disappeared and are replaced by connective tissue. In many tubuli there are hyaline or granular casts which stain red or blue by the azan stain. The interstitial tissue is considerably increased in amount and forms a network between groups of tubuli and glomeruli. In some places there is an abundance of distended capillaries and one has the impression that there is not only a dilatation of peritubular capillaries because of atrophy of the tubuli, but also a new formation of vascular channels. The interstitial tissue is more cellular in the cortex than in the medulla. The cells are large mononuclear cells, frequently with a foamy cytoplasm, lymphocytes, some tissue mast-cells and a few fibroblasts. The latter are more numerous in the medulla than in the cortex. Collagenous fibres are scanty. The interlobular and afferent arterioles show moderate media hypertrophy, occasionally splitting or duplication of intimal elastic membrane and very occasionally intima proliferation. Prisms and needles of birefringent material are present in moderate numbers. They form clusters in the interstitial tissue, mainly in the cortex and in the fibrous renal capsule. Occasionally, a small crystal is seen in a glomerulus, but never in glomeruli with advanced hyalinisation, necrosis or fibrosis.

Spleen : The Malpighian corpuscles are small, due to atrophy of the lymphoid component. In some places a Malpighian corpuscle is recognisable only by the presence of an arterial fork which is surrounded by large cells, 20–30μ in diameter, with a small often eccentric nucleus and a foamy cytoplasm which stains sky-blue with azocarmine or Masson's trichrome. The red pulp is poor in blood, which is

found almost entirely in the venous sinuses, scarcely at all in the meshes of the pulp. The cords of Billroth are very cellular and consist almost entirely of cells 20–30μ in diameter with a small, dark nucleus, with a pale nucleus, or without a nucleus. The cytoplasm is foamy, pale blue in azan and trichrome preparations. The littoral cells of the venous sinuses, wherever recognisable, do not show the foamy cytoplasmatic change. In comparison with the red pulp only small numbers of foam cells are seen in the trabeculae. Some of the trabecular and most of the follicular arteries show a marked thickening and hyalinisation of the walls. The severest changes in the arterial walls are seen in those follicles in which the lymphocytes have been completely replaced by foam cells and in those trabeculae where an appreciable number of foam cells is seen in periarterial arrangement. There are abundant birefringent prisms, needles and small hexagonal plates in the Billroth cords of red pulp, around follicular arteries, along the trabeculae, but only few within the trabeculae. They form clusters everywhere and in many places their intracellular situation is clearly recognisable. Occasionally a few free cells with intracellular crystals are seen in the lumen of a venous sinus, but only quite exceptionally is crystalline material seen in a littoral cell.

Liver : The lobular structure is well preserved and most of the cells show the normal radiating trabecular arrangement. In some places, however, the trabecular structure of part of a lobule is disorganised, with short rows of liver cells in various directions. Most of the liver cells are atrophic and show mild, fatty changes. Areas in which the cytoplasm of liver cells is very pale pink in haematoxylin-eosin preparations alternate with areas in which the staining is deeper. These show brick-red in azan-stained sections, while the former show pale brownish-yellow. There are small scattered areas of necrosis (50–70μ in diameter), in which aggregates of mononuclear and polymorphonuclear leucocytes are found. Most of these necroses are

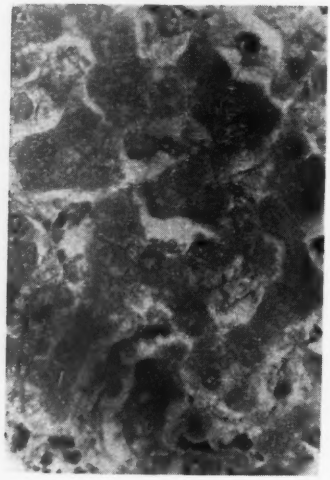


Fig. 22. Case 11. Liver. Azan. ×625, linear reduction $\frac{1}{2}$.

in the intermediate zone ; they are rarely seen close to the central vein and never in the periphery of an acinus. The liver cell trabeculae are widely separated, but this is not due to capillary dilatation, but to distension of Disse spaces. Narrow capillaries outlined by reticulum fibres are often bordered on each side by a space as wide as the lumen of the capillary. In these spaces, sometimes projecting into the lumen and occasionally within the lumen, cells of 20–25μ in diameter are seen with a small nucleus in foamy cytoplasm, pale blue in azocarmine stain (Fig. 22). The periportal spaces are not widened and show only a slight increase in cellularity. Scattered throughout the liver are clusters of birefringent crystals. These are grouped around one or a few nuclei and are situated between the liver cells, often within the distended Disse space. Not infrequently a single Kupffer cell with a few crystals can be seen and in such places there can be no doubt about the intracellular localisation. Only scanty crystalline deposits are seen in periportal spaces. As in the spleen, the number of foam cells seen in azan preparations exceeds the number of cells containing crystals in material fixed in alcohol and stained with basic fuchsin.

Lymph Nodes (upper deep cervical, tracheo-bronchial, mesenteric and peripancreatic) : Nests and strands of large, foamy cells are seen throughout the medulla and medullary cords and, to a lesser extent, in the centres of cortical follicles. The marginal sinus is distended and contains small cells with a dark nucleus. Many lymph sinuses of the cords and medulla are also distended and in some a few foam cells resembling those outside the sinuses are seen. No foam cells are seen lining the lymph sinuses. Clusters of birefringent crystals are abundant and correspond approximately to the distribution of foam cells as seen in azan-stained sections. The tracheo-bronchial lymph nodes show partial anthracosis and there is a clear relationship between crystals and pigment, both being seen in the same cell.

Thyroid : The vesicles are large, distended with colloid, and their epithelial lining is cuboid or flat. The interstitium is broadened and contains a fair number of foam cells which are elongated and have a fusiform nucleus. Crystals are rather scanty and foam cells without crystals are seen in sections from alcohol-fixed material stained with basic fuchsin. No crystals in the lining epithelium of vesicles.

Pancreas : The structure, the appearance of secretory cells and islands and the amount of interlobular connective tissue are all normal. There are only few birefringent crystals in ovoid cells of the interstitium and in the peripancreatic fat tissue where they are seen within fusiform cells between fat cells.

Heart : Scanty birefringent material in the subepicardial fat tissue and in the interstitial tissue of the myocardium, but no crystals resembling those found in other organs.

Skin : Structure normal. Very scanty and small birefringent crystals in the cutis vera and subcutis.

Suprarenal : Structure normal. Clusters of typical birefringent crystals abound in the medulla and the inner part of zona reticularis between the cortical cells. They disappear rapidly in the zona fasciculata and glomerulosa but increase again in the capsule and in the surrounding fat tissue.

Tonsil : Desquamated epithelium in the crypts, but no inflammatory changes. Abundance of typical birefringent crystals in the reticulum between the lymph follicles and in the connective tissue septa, but more within the follicles.

Parotid Gland : Structure normal. Fair number of clusters of typical crystals in the interlobular tissue and intralobular interstitial tissue, none in epithelial cells.

Aorta : Few birefringent crystals exclusively in the adventitia, otherwise nil abnormal.

Testicle : Immature testicle, but lumina of seminiferous tubules distinct. Many intracellular birefringent, small prisms in the interstitium, none in the seminiferous tubules.

Eye : There are few crystals in the cornea, sclera, choroidea, iris, none in the stroma of ciliary body and most in the subepithelial connective tissue of the limbus corneae, where they are frequently arranged in short rows along which rows of fusiform nuclei may be seen.

Brain : There is a fair number of crystals in elongated and ovoid cells of the connective tissue of choroid plexus. No crystals are seen in its epithelial lining. The plexus is oedematous. The brain shows hyperaemia, otherwise nil abnormal.

Spinal Cord : Normal.

Vertebrae : The columnar zone of cartilage is regular and not penetrated by vessels from the marrow. The provisional calcification zone shows scanty deposits of lime salts separated by areas without calcification. The primary trabeculae are short, and trabeculae perpendicular to the long axis are seen in the zone of primary marrow formation. The structure of the cancellous bone is dense and its trabeculae show broad osteoid seams (Fig. 23). The marrow is actively haematopoietic with a moderate number of fat cells. There is no fibrous marrow nor increased number of osteoclasts.

Proximal end of humerus : The articular cartilage is normal. The epiphyseal ossification centre shows marked osteoporosis and at the medial margin islands of cartilage are seen. The bone trabeculae show little calcium. The marrow is

Fig. 23. Case 11. Vertebra. Broad osteoid seams. Partial decalcification. H.E. ×48, linear reduction $\frac{1}{2}$.

fat with few small islands of haematopoietic tissue. The columnar zone of the epiphyseal cartilage plate is 250μ in thickness. The preparatory calcification zone is interrupted by broad tongue-like projections of fibrovascular tissue from the metaphysis. Within the zone of primary marrow formation and the metaphysis the marrow is almost entirely fibrovascular and there are numerous osteoclasts present. There are many Howship's lacunae but little dissecting bone resorption and few compound bone trabeculae. The osteoblasts vary in height, the majority are cubical, some are flat and some columnar. Towards the diaphysis this marrow is followed by a fat marrow with few small islands of haematopoietic tissue. Further distal the haematopoietic tissue prevails over the fat tissue. All bone trabeculae are poorly calcified and have broad osteoid seams or consist entirely of osteoid. With periodic acid-Schiff's reagent the cartilage gives a strongly positive reaction, while the bone trabeculae with the exception of a few centrally situated patches show a negative reaction.

Rib : The columnar zone of cartilage is rather narrow, 28μ in thickness. The provisional calcification zone and the marrow within the zone of primary marrow formation show the same changes as in the humerus, but the zone of fibrous marrow with numerous osteoclasts is narrow and immediately followed by a cellular haematopoietic marrow. However, the bone trabeculae are thin everywhere, with an increased number of osteoclasts and Howship's lacunae. The periodic acid-Schiff's reagent stain results in deep red staining of a cartilage and cartilaginous inclusions of the bone trabeculae ; the trabeculae themselves stain pink.

No **parathyroids** were examined histologically.

Case 15

R.B., sister of Case 16. Normal development until the time of weaning at the age of 7 months when she started vomiting and became constipated. Thought to be blind from birth. She gained weight poorly and developed a febrile disease with vomiting, extreme dehydration and gingivitis at the age of 2 years 8 months, of which she died 48 hours after admission. The autopsy was performed by Dr. White, Royal Infirmary, Cardiff, and revealed as principal findings minute white dots on liver, spleen, kidneys, lymph nodes, bone marrow, mesentery and mucosa of the duodenum. There was moderate dwarfing, dehydration of the skin, enlargement of the liver and to a lesser degree of the spleen and abdominal lymph nodes. The parenchyma of the kidney was generally pale. The femur showed no macroscopical evidence of rickets.

Histological Examination (only formalin-fixed material available)

Kidney : The glomeruli are either bloodless or they contain little blood. In most of the glomeruli, however, a few patent capillaries can be found. The majority of the glomeruli show a high cellularity. The epithelium of the visceral layer of Bowman's capsule is conspicuous, cubical, like that of a young infant. The high cellularity is due mainly to endothelial cells, but there are also large cells between the capillaries, with a kidney-shaped nucleus or multinuclear, and showing a foamy cytoplasm in azan stain. In some glomeruli there are patches where the nuclei are very poorly stained and occasional anuclear foci of necrosis. Such necrotic foci stain pale yellow-brown with van Gieson's stain. Occasionally one sees a few swollen epithelial cells of the visceral layer of Bowman's capsule with one or more hyperchromatic nuclei, but there are no capsular adhesions nor epithelial crescents.

There is a marked thickening of the basement membrane of Bowman's capsule, either circular or more often in the form of a crescent, particularly opposite the vascular pole. The thickening of the basement within the tuft is less severe and irregularly distributed. With periodic acid-Schiff's reagent a separation of the capillary from the epithelial basement membranes is often seen and a large cell with a vesicular nucleus may be seen between the two. Sometimes the basement membrane appears split into several thin P.A.S. positive fibrils. There is some pericapsular hyalinisation and pericapsular fibrosis, but no completely hyalinised or fibrosed glomeruli. The segments of tubuli are still recognisable, but the epithelium of proximal convoluted tubuli shows hydropic changes, the brush border (best demonstrable by the McManus-Hotchkiss technique) is absent, the lumina are dilated and filled with a granular eosinophilic material and occasionally a few epithelial cells are necrotic. The changes in the Henle's loops, in distal convoluted and collecting tubuli are less severe and there is frequently evidence of regeneration. All tubuli are separated by broad bands of loose connective tissue which shows a focal infiltration with large mononuclear cells and lymphocytes. Everywhere in the interstitial tissue large mononuclear cells with foamy cytoplasm are seen. In the subcapsular area of the cortex many tubuli have disappeared and are replaced by fibrous tissue so that the glomeruli are closely packed. The afferent arterioles and interlobular arteries show marked myoelastic hypertrophy, but no intima proliferation. Crystals are very scanty in the kidney. They form irregular plates with rounded edges, short prisms and sometimes aggregates of prisms arranged radially. They show marked dichroism of polarisation. Notwithstanding the large number of foam cells in the interstitium, crystals are only seen in the lumina of tubuli. A moderate number of tissue mast cells is seen in the interstitium.

Spleen : The lymphoid tissue of Malpighian corpuscles is atrophic. The follicular arteries are surrounded by broad rings of foam cells. These cells have a diameter of 15–25μ and their nuclei are vesicular or pyknotic ; some of the cells are anuclear, or nuclear debris may be seen scattered throughout the cytoplasm. The latter is pale blue and foamy in azan stain. Where a follicular artery is cut longitudinally a well demarcated strand of foam cells is seen accompanying the artery. Similar strands are also seen along penicillary arteries as they enter the red pulp. Within the pulp foam cells are less numerous. They are scattered either as single cells or as small clusters within the cords of Billroth or form curved bands around a Malpighian corpuscle. The venous sinuses are moderately wide, they contain red blood corpuscles, their endothelial lining is normal. Reticulin fibrils are seldom seen in close relationship to foam cells with Laidlaw's reticulum stain. Usually an island or strand of foam cells is surrounded by normal fibrillary splenic reticulum while only widely scattered, often fragmented argentophil fibrils are seen within the accumulation of foam cells. Only exceptionally are a few foam cells seen in the adventitia of a trabecular artery. The splenic blood vessels show no pathological changes. Birefringent crystals are abundant, but only in the cytoplasm of foam cells. The intracellular localisation of crystals is more clearly seen in the spleen of this case than in any other specimen.

Mesenteric Lymph Node : The marginal sinus is distended, but there is only a moderate number of cells within its lumen. The majority of these are small reticulum cells and only an occasional one is larger and has a foamy cytoplasm. The cortical follicles are small and show no definite germinal centres. The lymph sinuses of cortex and medulla are normal in width while the reticulum between the sinuses is

increased in amount. Within this reticulum are many cells 15–25μ in diameter with a foamy cytoplasm. Their nuclei are either pale vesicular or small hyperchromatic. Occasionally there is also an anuclear clump of foamy cytoplasm. With Laidlaw's reticulum stain some of the cells are seen outlined or bordered by argentophil fibrils while others show no relationship to fibrillary reticulum. A few littoral cells are swollen and have a foamy cytoplasm, and occasional foam cells may be seen free in a lymph sinus. Birefringent crystals are present in a moderate amount, considerably less than the number of foam cells.

Liver : The liver cells show severe fatty changes. The intralobular capillaries are narrow and the majority empty. The Disse spaces are distended and frequently the capillary is pushed towards one of the opposite trabeculae of liver cells. Within the Disse spaces here and there swollen Kupffer cells with a foamy cytoplasm are seen, but more frequently there are intralobular clusters of large foam cells, up to 30μ in diameter. Within such a cluster a capillary is not recognisable and reticulin fibrils are seen mainly in the periphery with a few fragmented fibrils within the cluster. Many of the foam cells forming such a cluster have a disintegrated nucleus or are anuclear. The periportal spaces show an increased cellularity with a fair number of foam cells which are usually smaller than those in the intralobular clusters. Small birefringent prisms and more or less well-formed hexagonal plates are present in a moderate amount. They are seen in the clusters of foam cells along intralobular capillaries and in periportal spaces. Their intracellular localisation is usually well recognisable.

Pancreas : Structure normal. There is some birefringent granular material, but no crystals resembling those in spleen and liver.

Heart Muscle : There is some oedema of interstitial tissue. Scanty birefringent material shows no resemblance to crystals in liver and spleen.

Lung : The air vesicles are poorly distended by air. There is a cellular infiltration of interalveolar septa and peribronchial tissue with large mononuclear leucocytes and lymphocytes. No birefringent material seen.

Tongue and its Mucous Salivary Glands : Nil abnormal, no crystals.

Parathyroids : Half of the neck organs (one side) were embedded and step serials prepared. Two parathyroids found, one 3.7×2.3 mm., the other 2×1.25 mm. in size. The larger was closely attached to the thymus (superior). Their structure is compact in the periphery and shows an arrangement of cords in the centre. The majority of cells are cloudy chief cells, the minority water clear cells. A moderate amount of fat cells is present in each parathyroid.

Thyroid : Normal in structure ; no crystals seen.

Stomach : The parietal cells are approximately normal in number, but there is evidence of regeneration in the presence of mitoses and binuclear forms. No crystals seen.

Intestine : Marked post mortem changes. However, several bottoms of Lieberkühn's crypts are well preserved and no Paneth cells can be seen.

Bones : Only the cortex of the shaft of femur is available. This has the structure of Haversian bone. There are many reversion lines with a fair number of osteoclasts and Howship's lacunae.

Case 16

D.B., brother of Case 15, died at the age of 6½ years. At 15 months anorexia, constipation, thirst and polyuria developed. In the years that followed the child became increasingly dwarfed and developed knock-knees. He was fair-haired and moderately photophobic. Repeated hospital admissions revealed renal glycosuria, albuminuria, hyaline and granular casts, red and pus cells in the centrifuged urine deposit. BP 85/60. At 2 years old Mantoux 1 : 1000 negative ; no later mention of tuberculin tests in notes at our disposal. Blood urea 131 mgm. per 100 ml., serum calcium 8.8, inorganic phosphate 5.5 mg. per 100 ml., alkaline phosphatase 62 units, plasma CO_2–combining power 21.8—29.1mEq/l plasma potassium 3.1 mEq/l (flame photometer estimation by Barclay). Cystine crystals were found in eyes by slit-lamp investigation and in bone-marrow. Urine chromatography (by H.B.) revealed a strong aminoaciduria with a pattern typical of Lignac-Fanconi disease. Microbiological assay (by H.B.) showed a tenfold increase over the normal in the urine concentration of valine, threonine and phenylalanine. Therapy with Albright's citrate solution, calcium phosphate and large dosage of Calciferol (100,000 units daily) was started at the age of 6. After three months the Calciferol dosage was reduced to 50,000 units orally daily and this was continued for five months until ten weeks before death, when Calciferol therapy was stopped.

Two months before death the boy became increasingly anaemic and the liver was noticed to be 2 ins. below the costal margin ; spleen just palpable. One month later he became drowsy, vomited blood and was admitted to hospital with severe manifest tetany, which was, however, soon overcome by treatment with calcium i.v. and paraldehyde.

C.S.F. clear, less than 5 cells per c.mm. Dull percussion note and fine crepitations over the lower lobe of left lung. The tetany was possibly due to a mistake in the dosage of Albright's solution (1½ instead of 1 oz. q.i.d.). In the following weeks hallucinations, diarrhoea, headache, haematemesis and Kussmaul's breathing were recorded, he became very drowsy, apathetic and finally lapsed into coma, from which he did not recover.

Investigations six weeks before death : serum calcium 12.4 mg.%, inorganic phosphate 10.3 mg.%, alkaline phosphatase 19 units. In the urine traces of albumen, nil abnormal in deposit.

Four weeks before death : serum CO_2—combining power 24 and 20mEq/l, calcium 10.8 mg.%, phosphate 12 mg.%, sodium 137 mEql, potassium 6.8 mEq/l. blood sugar 242 mg.%, blood urea 180 mg.%. In faeces an occasional R.B.C., no pathogens.

Three weeks before death : haemoglobin 7.7 g.% (53%).

Two weeks before death : Hb. 13.4 g.% (92%), after transfusions. Blood urea 468 mg.%.

Post mortem examination carried out between 50 and 60 hours after death by Dr. Parker, Royal Infirmary, Cardiff. The length of the body was 3 ft. 7½ ins. Post mortem lividity was not present ; rigor mortis was present. The general nourishment was poor. The eyes were equal and cadaveric. There was no evidence of disease or injury on external examination.

Heart : Weighed 88 g. (normal 90 g.). The left ventricle measured 10 mm., the right ventricle 4 mm. The pericardium, myocardium and valves, the mural endocardium, coronary arteries, aorta and other vessels appeared normal. There was a little muco-pus in the **left bronchus**. There was a small recent adhesion

on the posterior aspect of the right pleura and a few tubercles were present in the corresponding area of costal pleura. The **right lung** weighed 140 lg. The lung was congested and there was a caseous tuberculous primary focus 1 cm. across in the right upper lobe posteriorly. There were a few tubercles in the costal pleura on the left side. The **left lung** weighed 140 g. and was congested. Millet-sized tubercles were also present in the diaphragmatic pleura.

Gastro-intestinal Tract : The mouth, pharynx and oesophagus were normal. On the peritoneal surface of the diaphragm there was a large confluent mass of tubercles, but none were visible elsewhere. The small and large intestine and the stomach appeared normal. The **liver** weighed 740 g. (normal 630 g.). It was pale. There were 20–30 tubercles to be seen in the capsule and a few on the cut surface. On examination with a lens minute white dots could be clearly differentiated from tubercles. The **gall bladder** and **bile ducts** and the **pancreas** appeared normal. The **spleen** weighed 46 g. (55 g. normal). It was normal in colour, but the Malpighian corpuscles were not prominent. There were a few tubercles present. Some of the mediastinal and supraclavicular glands were caseous. The abdominal, inguinal and axillary glands were normal. The bone-marrow of the **right femur** appeared normal. The **thyroid** appeared normal. The right adrenal weighed 2.8 g. and the left 3.0 g. The adrenals and the pituitary appeared normal. The **right kidney** weighed 19 g. (normal 65 g.). The **left kidney** weighed 25 g. (normal 65 g.). Both were small with an increase in pelvic fat. The cortico-medullary demarcation was indistinct and the cortex was narrowed. The cut surface was pale with numerous yellow flecks, more prominent in the cortex. The capsule stripped easily, leaving a few coarse scars on the surface. The pelvis appeared normal. The bladder, genital organs and prostate appeared normal.

The **brain** weighed 1,420 g. and was normal. There was a subdural haematoma on the right side covering nearly the whole of the surface of the cortex of the right side. It was in the stage of pachymeningitic membrane formation and a cyst filled with thin fluid and altered blood. Vessels, spinal cord and nerves were normal. In the dura mater there were conspicuous white plaques.

Histological Examination

Kidney : The architecture of the kidneys is completely destroyed. There is a diffuse fibrosis of varying density. There is scarcely a normal glomerulus present. A few show only bloodless capillaries of the tuft with endothelial proliferation, the majority show focal necrosis, complete necrosis, hyalinisation and fibrosis. Hyalinisation and fibrosis are found mainly at the periphery of the tufts and there is a marked onion-peel periglomerular fibrosis. Some glomeruli show a proliferation of the epithelium in the parietal layer of Bowman's capsule. The tubuli are either atrophic or dilated and they are lined by cubical or flat epithelium. A differentiation of the segments is impossible. The dilated tubules are filled with pale eosinophilic material. There is a widespread focal round cell infiltration of the interstitium. Tissue mast cells are present in moderate numbers throughout the interstitium, independently of the foci of round cell infiltration. Crystals are seen in cortex and medulla, more in the latter. Here and there anisotropic crystalline material is seen in the glomeruli. A peculiar feature of this case is the extensive deposition of calcium salts, which are amorphous and stain typically with haematoxylin and with Kossa's reagent. They are mainly in necrotic tufts and a whole glomerulus may be replaced by a granular mass of calcium salts. Calcium deposits

are also present in the epithelium of Bowman's capsules, in basement membranes, in necrotic tubular epithelium and in walls of intralobular and afferent arterioles.

Spleen : The Malpighian corpuscles are atrophic ; the Billroth's cords of the red pulp are broad and contain large cells with a foamy cytoplasm and a small dark nucleus. These foamy cells are diffusely spread throughout the pulp ; nests are seen mainly around follicular arteries (Fig. 24) and along trabeculae. Here and there a trabecula is seen which consists almost entirely of a trabecular artery surrounded by foam cells. The venous sinuses are narrow and only few of the littoral cells have a foamy cytoplasm. Crystals abound and their distribution corresponds to that of foamy cells. In addition there is an abundance of haemosiderotic pigment. The latter is frequently seen in the same cells as the crystalline material, but the endothelial cells of the venous sinuses often contain haemosiderin only. There are a few epithelioid cell tubercles in the spleen with more or less advanced fibrotic changes.

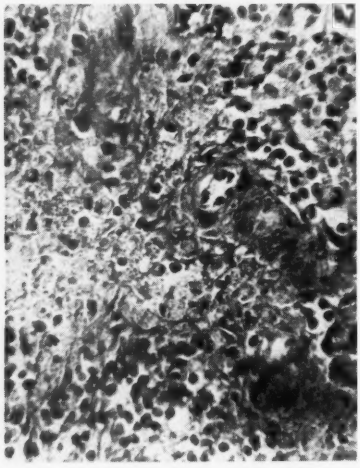


Fig. 24. Case 16. Spleen. Azan. ×380, linear reduction $\frac{1}{2}$.

Liver : The liver cells at the periphery of the lobuli show severe fatty infiltration. Scattered throughout the lobules, but more in the intermediate and central zones than in the peripheral, are groups of large spherical cells with a foamy or finely granular cytoplasm. The latter stains pale blue with azan and is almost colourless in haematoxylin-eosin. The size of the cells is 25–30μ. Many have no nucleus and are recognisable as cells only by their outlines, while others have a small, round, hyperchromatic nucleus. These clusters of cells usually fill the whole space between the liver cell trabeculae, but occasionally a compressed capillary is recognisable, shifted towards one of the liver cell trabeculae, particularly with reticulum stain. Apart from the formation of such clusters there is no marked swelling of Kupffer cells or distension of Disse spaces. Sections fixed in alcohol and stained with basic fuchsin show masses of birefringent crystalline material, which in

distribution corresponds to the clusters of foamy cells. A little crystalline material is present in the periportal spaces. The liver cells contain no crystals, but show either fatty infiltration or an abundance of glycogen granules. A few periportal spaces show a marked increase of fibrous tissue within which a small, isolated group of liver cells is sometimes seen. Apart from these changes there are epithelioid cell tubercles with large areas of caseous necrosis.

Stomach : There are abundant parietal cells in the fundus glands. The lumina of the glands are filled with a calcified material which stains typically with haematoxylin and with Kossa.

Small Intestine : Only an occasional Paneth cell was seen. There are many goblet cells in the epithelium.

Large Intestine : Apart from the presence of crystals no pathological changes. Crystalline material is seen in the wall of the whole gastro-intestinal tract, in the lamina propria, in the submucosa and subserosa, and to a lesser degree between the fibres of muscular coats. Most of the crystals are present around the bottoms of glands and close to the muscularis mucosae.

Lymph Nodes : One tracheo-bronchial lymph node shows extensive caseous necrosis with calcium deposits, but no crystals. All the other lymph nodes examined are overcrowded by crystals, mainly in the perisinusal reticulum cells of the medulla and medullary cords. They are almost absent in the littoral cells of the marginal sinuses and scanty in the lining of lymph sinuses of the medulla. Many lymph sinuses are, however, dilated and contain a fair number of free crystals. A peculiar feature of the sections fixed in formalin and stained with haematoxylin-eosin is that almost all crystalline material between the sinuses has disappeared and foam cells are seen instead, while the free crystals within the sinuses have persisted.

Lungs : One completely caseated focus 7 mm. in diameter with extensive deposits of lime salts, surrounded by a fibrous capsule. In the vicinity a few epithelioid cell tubercles with fibrotic changes. Areas of collapse and desquamative alveolar catarrh. A few small areas of bronchopneumonia. Moderate number of crystals in peribronchial and periarterial tissue, occasionally in interalveolar septa.

Pancreas : Slight dilation of acini ; their lumen filled with pale eosinophilic material ; their epithelium flattened. Moderate number of crystals in the interstitial tissue.

Suprarenals : Fair number of crystals between the cells of the cortex. It is not possible to make out whether the crystals are in the lining endothelium of capillaries or in cells between capillary and epithelial cell. Fair number of crystals in histiocytes between lobules of periadrenal fat tissue and in the suprarenal capsule.

Pituitary : Normal, apart from a cyst 5 mm. in diameter, lined by ciliated epithelium, in the posterior part of the anterior lobe, and a moderate number of crystals in the cells of the interstitial tissue of anterior lobe.

Brain : The villi of choroid plexus are oedematous and there is abundant intracellular crystalline material in the stroma, but none in the epithelial lining nor in ependymal cells. Otherwise the brain is normal.

Dura Mater : Within the macroscopically visible plaques there is a fair amount of crystalline material in elongated fusiform cells and also in the mesothelial cells.

Thyroid : Moderate number of crystals in histiocytes of the interstitial tissue ; otherwise nil abnormal.

Parathyroids : The whole neck was cut in serial sections, the cranio-caudal diameters of parathyroids were calculated from the number and thickness of

sections, the dorso-ventral and transverse diameters were measured microscopically on sections in which these diameters were largest. Approximately 20 per cent must be added to these measurements to offset the shrinkage in fixation and dehydration. The parathyroids found were the right upper, right lower and left upper. The measurements are as follows :

Parathyroid	Cranio-caudal	Dorso-ventral	Transverse
	mm.	mm.	mm.
Right upper ..	4.425	4.25	2.5
Right lower ..	5.25	5.0	2.25
Left upper ..	2.3	2.25	5.0

All parathyroids are definitely enlarged, the maximal diameter at this age being 2.5–3 mm. The structure is mainly compact, here and there with small lumen acini. The predominant cell is the water clear chief cell with a diameter varying between 10 and 20μ. The cloudy chief cells are scanty and eosinophil cells are only occasionally seen, as is usual at that age. Fat cells are absent within the parathyroids.

Bone-Marrow of Femur : The relation between active marrow and fat tissue corresponds approximately to the child's age ; cellular composition normal. For the histological findings on bones see page 221.

Discussion

A survey of the post mortem findings reported above shows that deposition of cystine crystals is the only constant and specific finding in Lignac-Fanconi disease. With the exception of Case 7, where crystals morphologically suggestive of cystine (Fig. 3) were found only in bone marrow aspirated *in vivo*, crystals identified as cystine were present in all or most organs, though their amount varied considerably. When abundant, such crystals are easily found in formalin-fixed material even in routine haematoxylin-eosin stain. But where crystals are scanty, as they are in the lungs, they may be dissolved when routine fixatives and stains are used. The quantity of crystals found in material fixed in alcohol was always conspicuously larger than in that fixed in formalin or Orth, although precautions had been taken against the acidification of the formalin. For this reason in every case where cystinosis is suspected part of the material should be fixed in absolute alcohol and stained with a solution of 0.5 g. basic fuchsin in 100 ml. of 50% alcohol. In an organ where cystine crystals are scanty, even after alcohol fixation they may be demonstrable only with basic fuchsin stain and not with haematoxylin-eosin. The same fixative and stain proved to be the most reliable for bone-marrow films. In the latter cystine crystals are not evenly distributed ; they tend to form aggregates at the margins of the film and in the tongue-like extensions which are frequently seen at the end of the film.

Even with alcohol fixation and basic fuchsin stain probably only a part of the cystine crystals present *in vivo* are preserved in the post

mortem material. This is suggested by the number of swollen foamy cells, which exceeds that of the crystalline deposits, and by the abundance of crystals seen in imprints from lymph nodes excised *in vivo*. It is, therefore, probable that within a short period after death changes take place (? in pH) which enhance the solubility of cystine.

The crystals may be identified by various methods. The most reliable of these is to compare the X-ray diffraction pattern of the material under examination with the X-ray diffraction pattern of pure l-cystine crystals. This has been carried out with the conjunctival biopsy of Case 8 (see Part 5) and with the spleen and liver of Case 9 (Fig. 25).

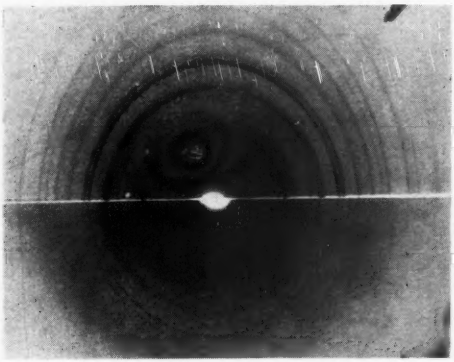


Fig. 25. X-ray diffraction pattern of pure cystine (top), and of spleen of Case 9 (bottom).

If a sufficient amount of crystalline material is present the tissues may be extracted with trichloracetic acid according to the **method of Nakamura and Binkley** (1948). For this purpose the tissues are ground in a mortar with a volume of 10% trichloracetic acid equal to the weight of the tissue. The suspension is then centrifuged and the supernatant fluid decanted. The extraction is repeated twice with half the volume of trichloracetic acid and the combined supernatant fluids are gently heated until the pH of the mixture is about 4. When this concentrated fluid is placed in a refrigerator a crystalline precipitate is formed within a short time. This precipitate consists mainly of hexagonal plates, especially after recrystallisation. They are indistinguishable from those seen in commercially obtainable cystine. They remain dark between crossed Nicols or show birefringence only at the margins. Prismatic birefringent forms do occur but they are scanty in the precipitate obtained by this method. When treated with phosphotungstic acid or phosphomolybdic acid according to the **method described by Bulloch and Kirk** (1935) tussles of long radiating needles are obtained. In this method a drop of concentrated sulphuric acid is added to a large drop of saturated phosphotungstic acid on a slide, and the

precipitate which is formed is redissolved by stirring or by the addition of a little water. This is diluted with 1–2 drops of distilled water and the crystalline material from the trichloracetic acid extraction is added. The mixture is heated almost to boiling point. The precipitate which forms after cooling is redissolved in a little water and recrystallised. The needles of cystine-phosphotungstate show, when examined between crossed Nicols and a sensitive tint quartz plate, a positive sign of elongation. The method of Bulloch and Kirk can also be applied to tissue sections, but usually, apart from the formation of long needles of cystine phosphotungstate or molybdate, cystine recrystallises in an altered habit with hexagonal plates and cube-like forms. Unidentified crystalline material which shows "Maltese crosses" may also be seen. When the cystine phosphomolybdate is produced, both its crystals and those of the cystine become dark blue owing to the presence of reducing substances.

For the **Wollaston test** a drop of strong hydrochloric acid is introduced under the coverslip on a slide with a deparaffinised section in absolute alcohol. The cystine hydrochloride also forms tussles of needles not unlike those of phosphotungstate and phosphomolybdate.

The **nitroprusside test** is useful for tissue extracts. The characteristic purplish-violet colour of a positive reaction is obtained only with cysteine ; cystine must be reduced to two molecules of cysteine by sodium cyanide. The latter has very weak reducing properties and the tissue extract in 0.1 N.HCl should, after addition of a freshly prepared 5% solution of sodium cyanide, be allowed to stand for at least 10 minutes before a dilute solution of sodium nitroprusside is added. The reaction is unstable and the colour disappears within a few minutes. No positive reaction is obtained with tissue extracts from cystinosis without reduction by cyanide, indicating the absence of appreciable amounts of cysteine. A positive diffuse colour reaction was also obtained with tissue sections but only after treatment with cyanide for 10 minutes.

All these reactions should be carried out, none of them being absolutely specific. A definite identification of the cystine is possible by **bidimensional filter paper chromatography** with tissue extracts. This was carried out with extracts from liver, spleen, lymph nodes, kidneys and/or bone-marrow in Cases 1, 2, 5, 8 and 9 (Fig. 26). This method, however, only gives semi-quantitative results, comparing the colour intensity of the cystine spot (as cysteic acid) with that of a normal control. Accurate quantitative estimations are possible by **microbiological assay** and by the **resin exchange method** of STEIN and MOORE (1951). The former has not been used in our investigations because only fixed material was available at the time when the technique had been established in this department. With the resin exchange method Dr. Dent obtained the following figures :

Case 9 : Spleen, 934 mg. cystine/g. total nitrogen
Liver, 248 mg. cystine/g. total nitrogen

as compared with normal values of 1.5–2.5 for the spleen and 2–3 for the liver.

Sometimes the only available material for identification is a bone-marrow film or an imprint of a lymph node. In such a case the only method applicable is **optical crystallography**. This line of investigation

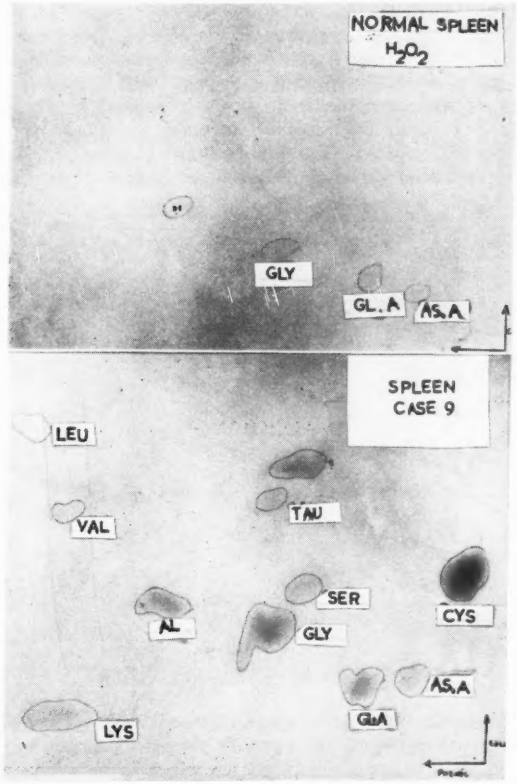


Fig. 26. Chromatograms of extract of normal spleen (top) and of spleen of Case 9 (bottom). Abbreviations of aminoacids, see Fig. 2, Part 1.

has not yet been fully applied to our material but some results obtained with the kind help of Dr. Lacey, from the Department of Geology, University of Birmingham, will be reported here.

Hexagonal plates characteristic of commercial crystalline cystine are not often seen in the tissue sections. Even when present a high degree of symmetry is only

exceptionally obtained and the majority of crystals are acicular or prismatic. Both vary considerably in length but short prisms prevail. Occasionally forms are seen which appear to be cubes. These always show a strong birefringence between crossed Nicols, thus proving that they are not true cubical crystals, as the latter are isotropic. Sometimes rounded edges indicating grossly underdeveloped faces (probably two) are recognisable. It is, therefore, very probable that such "cubes" represent side views of short hexagonal prisms with two grossly underdeveloped faces. A basal section of such a prism would resemble the sketch in Fig. 27 in the centre of a circle. A double projection in plane of the faces is made according to the principle of spherical projections used in crystallography (see PORTER and SPILLER, 1951). From the centre normals are drawn to the faces represented here by lines. The points of intersection of these normals with the circle, which has the same centre as the basal section of the crystal, are connected by straight lines and

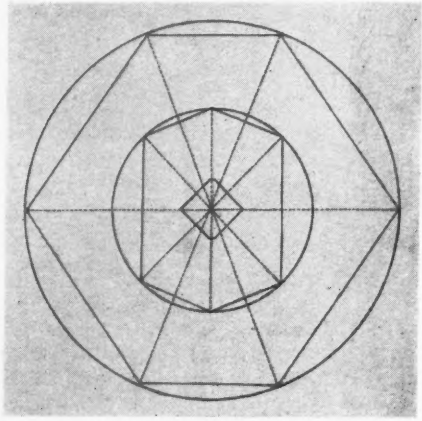


Fig. 27. Double bidimensional projection of a "pseudocubical" crystal.

the projection repeated on a second circle. It is seen how with each projection the semicubical crystal approaches asymptotically a symmetrical hexagonal shape.

The underdevelopment of faces is not the only reason why some crystals simulate cubes. In some cases such cubes show a pattern of zoning which clearly indicates that they consist of a number of prisms cemented together.

The medium in which cystine crystallises exerts a profound influence on the form of the crystals. Although they differ considerably both from the crystals of commercial cystine and those obtained by extracting tissues, it seems certain that the widely varying crystals seen in tissue sections are actually different forms of the same substance.

In order to prove that the marked difference between cystine crystals obtained from aqueous solutions and crystals seen in tissue sections is due to differences in the medium, pure cystine, which showed on microscopical examination only

hexagonal plates, was dissolved in weak ammonia solution ; when recrystallised the crystals were mainly flattened. A cystine solution was then added to 30% bovine albumin, 5% gelatine and 1% agar and left for a few days in the refrigerator. The deposit in bovine albumin was examined microscopically, while from the gelatine and agar frozen sections were prepared. In all three instances, but particularly in agar, there was a preponderance of acicular forms either identical with those seen in tissue sections or longer (Fig. 28). The habits of cystine crystallised from aqueous solutions are, therefore, characterised by a tendency to flattening, those of crystals formed in a colloidal medium and in tissues by a tendency to elongation.

All crystals in tissues of cystinosis show strong birefringence between crossed Nicols except the hexagonal plates. This is easily

Fig. 28. Cystine crystallised in agar. ×625, linear reduction ½.

understandable on the assumption that the crystals are all of the hexagonal system and therefore monoaxial. The hexagonal plates are basal sections, their plane is perpendicular to the long crystal axis and to the optical axis. The plane polarised light coming from the polariser passes through the optical axis and is not dissolved into an ordinary and an extraordinary ray. No light can, therefore, pass through the analyser and the field remains dark. There is no reason to assume that cystine is present in tissues in two forms, one amorphous and the other crystalline anisotropic (FANCONI 1946), nor that there are two crystalline substances, one with positive and the other with

negative anisotropism, one representing cystine, the other a cystine-peptide (BOEHNCKE, WEYERS and TEPE, 1952).

The determination of the optical sign of crystals found in the tissues is extremely difficult because of their minute size. Only on one occasion was it possible to visualise with the help of a Bertrand lens somewhat ill-defined interference figures. These were suggestive of negative anisotropism. Pleochromism of polarisation is frequently seen in the somewhat thicker crystals and occasionally even in acicular forms. An examination with the sensitive tint quartz plate showed some elongated crystals to be "long fast" and others "long slow." In monoaxial crystals the sign of elongation is identical with the optical sign of the crystals and thus it appears that the statement of BOEHNCKE et al. (1952) concerning the presence of crystals with negative and positive anisotropism is correct, but it is definite that, while the hexagonal plates are negatively anisotropic, among the elongated forms both positive and negative anisotropism occurs. This is, however, no evidence of the presence of two substances. Pure aminoacids may crystallise in different forms, not only habits (BERNAL 1931). As mentioned above we have obtained elongated forms of cystine crystals in 1% agar. These, when examined with the sensitive tint quartz plate, proved also to have either a positive or a negative sign of elongation.

Occasionally crystals in tissue sections or bone-marrow films show a zoning by narrow bands across prismatic or slightly bent forms. These bands are dark both in ordinary light and between crossed Nicols. Sometimes the outline of such a "prism" is not straight and particularly in the form in which it is slightly bent it resembles a rouleau of plates. Apparently this is due to imperfect crystallisation with inclusion of an amorphous matrix which cements plates of crystals together. The plates are in this case seen at right angles to the optical axis and are therefore strongly birefringent.

Cystine crystals have previously been confused with calcium. BENOIT (1935) concluded that in his case the crystalline material was calcium because of a positive Kossa reaction, although no staining with haematoxylin was obtained. ROULET (1941) also found some blackening of crystals with Kossa's method and suggested that this was due to the reducing properties of cysteine. We have never obtained a positive Kossa reaction with the crystalline material in Lignac-Fanconi disease. The behaviour of the crystals in the sodium nitroprusside reaction (see above) rules out the presence of any appreciable amounts of cysteine on the surface of cystine crystals. Minimal traces may be present, accounting for the occasionally positive Kossa reaction and possibly also for the blue discolouration of cystine phosphomolybdate crystals.

While detailed knowledge of cystine crystals as they occur in tissues is important for their identification, their **localisation** permits of definite conclusions as to the nature of the pathological process. From the description of our histological findings the following facts emerge :

(1) The cystine crystals are found exclusively in the reticuloendothelial system, never in parenchyma cells.

(2) They show preference for a certain part of the reticuloendothelial system ; the littoral cells are not usually involved, while massive cystine storage is generally found in Kupffer cells, splenocytes, histiocytes throughout the connective tissues of the whole body, and reticulum cells of Billroth cords in the spleen and of medullary cords of lymph nodes.

(3) The origin of the crystals is **intracellular** (see BAAR 1951). The intracellular situation of cystine crystals was also noted by ROULET, ESSER and BÜRKI (1941), and JAKOBSON in DRABLØS' paper (1951) mentions that the crystals were "in some places plainly intracellular."

It has been repeatedly stated or tacitly assumed that storage of the crystals in the reticuloendothelial system is due to phagocytosis. A thorough examination of many hundreds of sections has convinced us that this assumption is not correct. The apparently extracellular situation of crystals in bone-marrow films will be discussed below. In tissue sections the number of free extracellular crystals is negligible when compared with the masses of intracellular crystals and can easily be explained by the disintegration of the cells within which the crystals had formed. Within cells the crystals are mainly seen as closely packed clusters around a nucleus, as aggregates with definite outlines with the inclusion of a few nuclei, or as similar aggregates in which the nucleus has disappeared. The littoral cells have the highest phagocytic properties and yet they only occasionally contain crystalline material. This is best seen in the periphery of a lymph node, where the marginal sinus may be free of crystals, while in the vicinity masses of crystals are present in the reticulum cells around the lymph sinuses of medullary cords.

There is never a foreign body reaction around cystine deposits in Lignac-Fanconi disease. By contrast, when a solution of cystine was injected intramuscularly into rats, the reaction was conspicuous and consisted of a dense infiltration with lymphocytes, large mononuclear cells and polymorphonuclear leucocytes. The cystine crystallised in the tissues with the formation of a few hexagonal plates and a preponderance of elongated forms, prismatic or acicular. These were generally longer than those in tissues of Lignac-Fanconi disease but closely resembled the habits of cystine crystals in gelatine or agar. In guinea-pigs a single injection of a heavy suspension of cystine crystals in normal saline produced a typical granuloma with foreign body giant cells (see Fig. 29).

The localisation of cystine crystals in tissues in Lignac-Fanconi disease resembles that of kerasin in Gaucher's disease, although even

in the infantile form of Gaucher's disease the storage is less widespread. The cystine storage in the reticuloendothelial system may be diffuse as in Case 9, or in some organs there may be a formation of nests of greatly swollen cells which, after the cystine crystals have been dissolved, show a foamy cytoplasm. Such nests are not unlike the nests of Gaucher cells.

There follows an account of the histopathological findings in individual organs.

The **liver** constantly shows a swelling of Kupffer cells, which is associated with a distension of Disse spaces, particularly when the

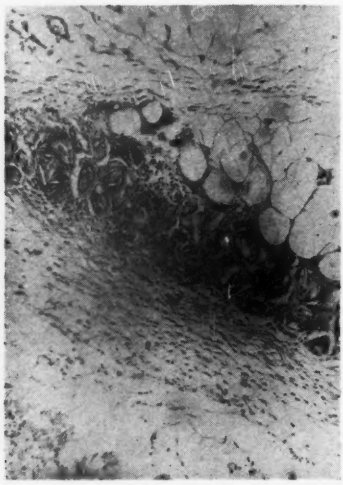


Fig. 29. Granuloma at the site of cystine injection (heavy suspension). ×120, linear reduction ½.

swelling is diffuse and not in the form of nests. The Kupffer cells usually remain in the Disse spaces, while the capillaries become compressed and separated from the parenchymal cells. This may account in part for the extreme paleness of the organ, though the widespread fatty infiltration of liver cells which is commonly seen may also contribute to the pale yellowish colour. Compression of capillaries leads to inadequate nutrition of liver cells and particularly to an inadequate supply of oxygen. Anoxia of liver cells is probably the main cause of the fatty infiltration commonly seen in Lignac-Fanconi disease, though complicating infection may be a contributory factor. These changes

and possibly a transudation of serous fluid into the distended Disse spaces (RÖSSLE 1930) will finally cause liver cirrhosis (Himsworth's diffuse hepatic fibrosis). Liver cirrhosis has been described in suspected cases of Lignac-Fanconi disease by VAN CREVELD (1934), by GUILD, PIERCE and LILIENTHAL (1937), and incipient cirrhotic changes were seen in our material in Case 16 (see page 202). From the clinical manifestations which they reported it is suspected that van Creveld's and Guild's cases are examples of Lignac-Fanconi disease, though that of van Creveld was clinically somewhat atypical and in none were deposits of cystine crystals found or mentioned.

The **spleen** may be enlarged or normal in size. Its consistency is firm. On the cut surface the Malpighian corpuscles appear atrophic and are sometimes scarcely discernible. The trabecular system, on the other hand, is always conspicuous. A characteristic though not invariable feature is a diffuse white stippling on a bright red or greyish red background, as if the cut surface were powdered with talc or flour. This appearance becomes spectacular when a lens is used for examination (see Fig. 30). On histological examination the swollen crystal-laden

Fig. 30. Case 16. Spleen.

Fig. 31. Cystine crystals in bone-marrow film of Case 8.

reticulum cells are seen mainly in the cords of Billroth. The venous sinuses are narrow and contain little blood. Their lining endothelium only rarely contains crystals. The small Malpighian corpuscles contain little crystalline material and this is found mainly around the follicular arteries. The number of crystals in the trabeculae varies but along their margins there is often a row of crystal-laden cells. Vascular changes are variable and not always present.

The **lymph nodes** always contain an abundance of crystalline material. Since lymph nodes are available as biopsy material for the diagnosis *in vivo*, in one case at the post mortem examination a series of lymph nodes from the cervical, axillary, mediastinal, mesenteric and inguinal regions were taken for imprints and sections. Some of these nodules were no more than millet-sized. All sections and imprints showed an abundance of crystalline material. *In our opinion a lymph node biopsy is the most reliable procedure for a definite diagnosis of Lignac-Fanconi disease in vivo.* Histologically the bulk of the crystals are seen in the reticulum cells around lymph sinuses. The marginal sinus, though distended, is usually free from crystals. The lymph sinuses of medullary cords may be free from crystals, but sometimes crystals are seen in the littoral cells and free in the lumen. These are more often shaped like hexagonal plates than are the intracellular crystals. It has been shown above that the habits of cystine crystals are determined by the medium from which the cystine crystallises. It is possible that with the disintegration of crystal-laden cells a concentrated solution of cystine also passes into the lymph sinuses, where it may crystallise extracellularly.

The most striking findings in the **bone-marrow** are (1) the abundance of crystals in sections and the comparative scantiness in films ; (2) the almost exclusively intracellular situation in sections and the extracellular situation in films. Obviously the findings in sections represent the true distribution. In spreading aspirated bone-marrow material many cells, and particularly the swollen and often anuclear cells, are mechanically disintegrated and the free aggregates of crystals (Fig. 31) tend to accumulate on margins and in the tongue-like extensions of the film. The number of crystals in sections from bone-marrow obtained at necropsy is usually greater than in sections from aspirated material, as some of the cystine appears to dissolve in the blood which is aspirated with the bone-marrow. With lymph nodes the reverse is the case. The crystals are present only in reticulum cells of the bone-marrow but never in haemic cells or osteoblasts and osteoclasts.

Table 1

CELLULAR COMPOSITION of BONE-MARROW in FIVE CASES of LIGNAC-FANCONI DISEASE

	Normal	Case 1 K.C.	Case 2 P.R.	Case 5 J.N.	Case 8 M.R.	Case 9 M.B.
Haemocytoblasts	0.5	0	0	0	0.2	0.5
Megaloblasts	0	0	0	0	0.2	0
Basophil macroblasts	0.1— 1.0	1.2	0	0	1.0	1.0
Basophil normoblasts	1.0— 5.0	0.6	2.5	0.5	1.8	4.0
Polychromatophil normoblasts ..	10.0—20.0	19.6	7.0	7.5	12.8	6.0
Orthochromatic normoblasts ..	0.0—10.0	1.4	2.0	0.5	1.4	0
Myeloblasts	1.0— 3.0	2.0	1.5	0.5	2.4	3.5
Promyelocytes	1.0— 3.0	2.6	2.0	1.0	1.0	2.5
Neutrophil myelocytes	5.0— 8.0	8.2	11.5	10.0	3.2	7.5
Neutrophil metamyelocytes ..	10.0—15.0	11.6	11.0	11.5	2.0	8.5
Neutrophil band forms	14.0—35.0	25.2	15.5	24.5	30.6	14.5
Neutrophil segmented	7.0—25.0	14.0	17.0	18.0	20.0	23.5
Eosinophil myelocytes	0.2— 1.5	0.2	1.0	0.5	1.2	1.5
Eosinophil polymorphonuclear ..	0.5— 4.5	0.6	5.0	3.0	0.8	4.0
Basophil myelocytes	0.0— 0.1	0	0	0	0	0
Basophil polymorphonuclear ..	0.1— 0.2	0	0	0	0	0
Large reticulum cells	0.5— 4.0	2.4	4.5	1.0	5.0	8.5
Plasma cells	0 — 2.0	0	0	1.0	0	0
Small lymphoid cells	3.0—25.0	10.2	19.0	20.5	8.2	14.0
Megakaryocytes	0.1— 0.3	0.2	0.5	0.5	0.4	0.5

The cellular composition of the bone-marrow shows no characteristic change (Table 1). Eosinophilia reported by ESSER (1941) was absent in all five cases examined. There is no significant increase in the number of reticulum cells, which is remarkable if the large number of crystal-laden cells seen in sections is borne in mind. It shows that these cells are easily mechanically disintegrated in the process of preparing a film.

Kidneys. The renal pathology in a full-blown case of Lignac-Fanconi disease is the most impressive gross anatomical finding. Nevertheless, and this is of paramount importance, verified examples of Lignac-Fanconi disease have been reported in which the kidneys showed no or only insignificant changes (HOTTINGER 1947, DRABLØS 1951). This is borne out by our Case 7, though the fact that in this case cystine crystals were found only in films of aspirated bone-marrow and none in the post mortem material makes its interpretation equivocal. As the kidneys of all our other cases showed advanced changes we have

supplemented our personal experience by using the reports of other workers in order to gain a better understanding of the natural history of the kidney lesion. Yet the definitely focal distribution of renal lesions, with the presence of a variety of stages in the same organ and even in the same section, permits some conclusions as to the evolution of renal and particularly glomerular pathology, and may be used to supplement the available descriptions of mild renal changes.

In the early stages the size of the kidneys is normal or enlarged, but in later stages it is considerably reduced and the surface often shows a uniform fine granulation. The colour at this stage is pale yellowish-white. Such a kidney combines the colour of a "large white kidney" with the size and surface granulation of a "genuine contracted kidney." The early histological changes are quite uncharacteristic, and the kidney may be reported as normal (HOTTINGER 1947, DRABLØS 1951), or there may be oedema of the interstitial tissue, granular degeneration of proximal convoluted tubules, occasional thickening of basement membranes and little blood in the capillaries of glomerular tufts. Oedema and swelling of basement membranes are sometimes demonstrable only with special stains, particularly silver impregnation (FUCHS and POPPER 1937) and McManus Hotchkiss' periodic acid-Schiff's reagent. Although this change, seen in Case 7, is common and trivial, it may proceed to severer changes of interstitial nephritis (ZOLLINGER 1945). In the case studied by WASER (1946), where the tubular changes were moderate and the glomeruli showed only variation in size and swelling of the mesangium (part of interstitial oedema) with narrowing of capillaries, there were already interstitial round cell infiltrates. The early changes of the tubular epithelium are described as granular swelling with "large albuminous granules" (LIGNAC 1924, Case 1), granular, fatty and vacuolar degeneration (WASER), or as cells which are "extremely large, practically blocking the lumen, but not the cloudy swelling type of enlargement" (GUILD, PIERCE and LILIENTHAL, 1937). The glomeruli are described in the early stages as normal, or there may be swelling of the mesangium (WASER, our Case 7), or the capillary tuft may contain scarcely any blood (LIGNAC, Case 1, our Case 7).

As the next stage we may consider the kidneys described by STURZENEGGER (1939). They were reported as having a smooth surface and a pale greyish-white colour with numerous minute yellowish dots. On section the separation of cortex from medulla was distinct and the same yellowish dots were seen as on the uncut surface. Histologically there was a hydropic-vacuolar degeneration of tubular

epithelium, and a few round cell infiltrates of the interstitium, while the glomeruli occasionally showed endothelial proliferation. Sturzenegger interpreted the round cell infiltrates as residuals of an old pyelonephritis, but in view of the constancy of such infiltrates in all more or less advanced examples of Lignac-Fanconi disease without clinical evidence of urinary tract infection we must consider the interstitial round cell infiltration as an integral part of the renal pathology in this disease. The renal changes in our Cases 4 and 15 represent intermediate stages between the above described initial alterations and the complete disorganisation seen in Cases 9, 11 and 16.

In all advanced cases a diffuse interstitial fibrosis, uneven in distribution and density, is the most conspicuous histological finding and is associated with atrophy and cystic dilatation of renal tubuli and with glomerular changes, which vary from minute focal necrosis to complete hyalinisation and fibrosis with atrophy. In large areas no tubuli are recognisable, in others there are cystic spaces lined by flattened epithelium and filled with a colloid-like material. The latter are indistinguishable from the pseudo-colloid cysts of chronic pyelonephritis except that there is no predilection for the boundary zone. Wherever tubuli are seen they are lined by a cubical or flat epithelium ; nowhere is a brush border present and it is impossible to differentiate segments of a tubule. CLAY, DARMADY and HAWKINS (1952) isolated individual nephrons by microdissection and showed that there was not only an alteration in tubular epithelium but also an abnormality of the first part of the convoluted tubule, which was shorter than normal. Apart from fibrosis the interstitial tissue shows variable degrees of focal round cell infiltration. This consists mainly of lymphocytes and large mononuclear cells, but plasma cells and polymorphonuclear leucocytes are occasionally also present.

A peculiar finding is that of fairly numerous tissue mast cells scattered throughout the renal interstitium. In the literature it is stated that mast cells do not occur in normal kidneys and we could not find any reference to mast cells in pathological kidneys. We have examined a series of kidneys from various diseases and only on one occasion, in a kidney with chronic pyelonephritis, did we find an occasional tissue mast cell.

There is no reasonable explanation for the finding of mast cells in kidneys of Lignac-Fanconi disease if only their heparin-forming function is taken into account. Recently, however, RILEY and LENDRUM (1952) reported observations suggesting that mast cells form the intercellular cement of connective tissue. They believe that mast cells have a formative or nutritive function in respect of collagen. This would make their presence in a primarily interstitial nephritis understandable.

The glomeruli show the greatest variety of changes. BEUMER and WEPLER (1937) found normal glomeruli in spite of advanced fibrotic changes and the presence of pseudo-colloid cysts. The usual finding in advanced cases is that some glomeruli are normal, some bloodless with swelling of endothelial cells, while others show a definite widening of the mesangium with splitting of the basement membrane. Severer changes are indicated by focal glomerulonecrosis. Such necrotic areas stain yellow or brownish with van Gieson. In glomeruli which have undergone complete necrosis the periphery stains pink, the centre yellow or brown. Proliferation of the epithelium of Bowman's capsule is not a conspicuous feature, though it does occur and multinuclear epithelial giant cells with hyperchromatic nuclei may be seen in the visceral layer (Fig. 10). Very occasionally in a degenerating glomerulus fibrils are seen which take Weigert's fibrin stain (Fig. 11) and such fibrils may be present also in the space of Bowman's capsule and in the periglomerular interstitium. Periglomerular hyalinisation and periglomerular fibrosis are a common finding in all advanced cases and precede the fibrotic atrophy of the glomerulus.

Cystine crystals are less abundant in the kidneys than in lymph nodes, bone-marrow, spleen and liver. They are seen mainly in aggregates within large cells and in the medulla more than in the cortex. Not uncommonly a few minute crystals are seen in the mesangium of a glomerulus and free in the space of a Bowman's capsule. Only exceptionally a lumen of a proximal tubule is packed with crystals which thus form a renal microlith (Fig. 13). Disintegration of mesangial cells could not account for such a mass of crystals and it is probable that we have here to deal with extracellular crystallisation from the glomerular filtrate after reabsorption of water and salts. It is well known that this reabsorption, though isosmotic, is selective (SMITH, 1951).

The phosphatase reaction was negative in all the kidneys examined. We were originally inclined to attribute some significance to this finding, particularly because it was not limited to the kidneys but was also seen in liver, spleen and bone-marrow, while the phosphatase reaction in the small intestine was normal (BAAR 1951). Further investigations have, however, shown that phosphatase disappears from all kidneys with chronic inflammatory changes, especially in chronic pyelonephritis.

Summarising, the kidney of Lignac-Fanconi disease can be classified as chronic interstitial nephritis with tubular degeneration. This term, once discredited because it included chronic pyelonephritis,

chronic haematogenous descending nephritis, some cases of chronic glomerulonephritis and arteriolosclerotic contracted kidney, was in our opinion properly reinstated by ZOLLINGER (1945). In the nomenclature of Russell the kidney of Lignac-Fanconi disease belongs to the group of nephritis repens. Although it has some peculiarities, such as early minute focal necrosis with late hyalinisation and fibrosis, it can definitely be differentiated from other forms of chronic interstitial nephritis only by the demonstration of cystine crystals.

An unusual finding not directly related to Lignac-Fanconi disease is the calcification in the kidneys of Case 16, the pathology of which is described on pages 201-202. The localisation of calcium deposits was entirely different from that seen in renal acidosis. In addition to nephrocalcinosis, there were deposits of calcium in the fundus glands of the stomach and also in walls of arteries. It is almost certain that these calcifications are the result of hypervitaminosis D, possibly with alkalosis as contributing factor, and although seen in only one case this observation should underline the importance of close biochemical control when massive vitamin D and alkali therapy are supplied.

In the **gastro-intestinal tract** one of us (BAAR 1951) has reported the atrophy of parietal cells in the fundus glands of the stomach and of Paneth cells in the Lieberkühn's crypts of the small intestine. Both findings proved to be inconstant. The first may account for the achlorhydria, which is also an inconstant finding. It was present in three of our patients and the one who died showed the above mentioned changes. The significance of the second finding is obscure. Cystine crystals, usually clearly intracellular, are found mainly in the lamina propria and in the submucosa. They may be abundant in the reticulum of the lymphatic apparatus and around the tips of the glands but are scanty in the connective tissue of the villi.

In the **central nervous system** the only significant pathological finding is the presence of cystine crystals in the stroma of choroid plexus. It is a matter of conjecture whether these are responsible for the neurological symptoms sometimes observed (VAN DER ZIJL and HESLINGA 1940, our Case 7) and whether they may be the cause of a complicating hydrocephalus or a predisposition to meningitis. LIGNAC's Case 3 (1926) had a hydrocephalus after meningitis and our Case 9 died of purulent meningitis. Plaques of cystine deposits in the dura mater were found in Case 16. A peculiar finding was that of crystals in the cells of mesothelial lining and also in fusiform cells which resembled fibroblasts but were probably histiocytes.

The presence of cystine crystals in the **conjunctiva** and **cornea** is of great diagnostic importance. The histological report on the biopsy specimen from Case 8 is included in Part 5. Unfortunately in the only case in which the whole eye-ball was removed at the post mortem examination a fixative which contained mercury was used and this combined with cystine to produce amorphous precipitates. We are therefore unable to make full statements about the distribution of cystine crystals in the eye. Histopathological findings on the whole eye are reported by BÜRKI (1941). The changes in the conjunctiva were identical with those reported in Part 6. Abundant crystalline material was present in the basal plate of the ciliary body and in the ciliary processes, less in the chorioidea. In the retina there were degenerative changes in the nerve cells, oedema in the layer of nerve fibres, and glia proliferation, but no crystalline deposits. Oedema and glia proliferation were also present in the optic nerve. In the vicinity of crystalline deposits of the uvea there was a slight round cell infiltration. No crystals were found in the lens nor in the vitreous body. Our findings in the conjunctiva biopsy specimen of Case 8 (Part 5) and in those parts of the eye of Case 11 which have been examined suggested strongly that all crystals were originally located in histiocytes.

As mentioned previously cystine crystals may be found in the **histiocytes of the connective tissue of any organ** of the body. In the lungs they are scanty, mainly in the peribronchial and periarterial interstitial tissue, only exceptionally in the macrophages of interalveolar septa. Crystals are also scanty in the interstitial tissue of the heart.

The microcystic dilatation of **pancreatic** acini and ducts in Case 9 is reasonably explained by the presence of uraemia (BAGENSTOSS 1948, a and b). A dilatation of pancreatic acini was also seen by KING and LOCHRIDGE (1951). In this case lack of vitamin A absorption was found *in vivo*.

The histopathology of **bones**, although after the renal pathology the most interesting aspect of Lignac-Fanconi disease, will be only briefly discussed. Detailed descriptions of findings in individual cases have been given above and are not only identical in all our cases but are also identical with those described by STURZENEGGER (1939), ROULET (1941) and LOOSER (1944). It must, however, be emphasised that the changes are essentially rachitic and that there is no feature of the histopathology of bones in Lignac-Fanconi disease which is not met with in rickets of different aetiology, particularly in the severer forms of

"rachitis tarda," with which fortunately only the older generation of pathologists is familiar. Excellent descriptions can be found in the monographs of SCHMORL (1909) and of SCHMIDT (1929).

Changes resembling those of osteitis fibrosa are not constant but they are not uncommonly seen in the metaphyseal areas of long bones. More frequently a fibrous metaplasia of the bone-marrow is found in these areas without increase in the number of osteoclasts or evidence of lacunar bone resorption. A constant finding in advanced untreated cases is a rachitic intermediate zone with widely separated patches of calcified cartilage, with islands of uncalcified cartilage within bone, osteoid or fibrous tissue or at least interruption of the provisional calcification zone and marked broadening of osteoid seams. Although observations of Lignac-Fanconi disease without clinical and radiological evidence of rickets are reported by FANCONI 1946, Case 3 ; FANCONI and BICKEL 1949, Case 2 ; KING and LOCHRIDGE 1951 ; MONOD 1951, we doubt whether this could be upheld if histological examinations were available in each case. In Fanconi's Case 3 WASER (1946) found histological evidence of healed rickets ; the cases of Fanconi and Bickel and Monod were still alive at the time of publication, while in King and Lochridge's case only a costo-chondral junction was examined. In the present series there are two cases (4 and 5) without clinical or radiological evidence of rickets. In one there was the history of rickets being diagnosed at the age of 6 months, while the other had a raised alkaline serum phosphatase and low serum phosphates. Further evidence that the bone changes in Lignac-Fanconi disease are essentially rachitic is provided by the histological findings in Case 16. Active rickets was diagnosed in vivo and the child was treated with massive doses of calciferol. Necropsy revealed an absence of rachitic changes and of osteitis fibrosa.

The question of osteoporosis and osteosclerosis in Lignac-Fanconi disease is of more than academic interest. Both are frequently reported, particularly osteosclerosis at the proximal end of the femur. It must be borne in mind that osteoporosis is not an essential feature of rickets, although it is a frequent accompaniment. The essential feature is failure of calcification in the preparatory zone of cartilage and the formation of uncalcified bone tissue, i.e. osteoid. The latter is more resistant to normal osteoclastic resorption than calcified bone (WEINMANN and SICHER 1947). Thus in rickets bone tissue is found which is denser than normal but not calcified, a condition which Erdheim used to call osteoid-sclerosis, and which in rickets is commoner

than true osteoporosis. It is doubtful if the radiologist can always clearly differentiate the two conditions. The excessive formation of osteoid takes place mainly in the areas of mechanical stress, and when in such a tissue calcification occurs as the result of therapy the bone will show true osteosclerosis.

Quite apart from any atrophy of disuse, true osteoporosis may also occur as a result of parathyroid hyperfunction. This is a common finding in any rickets of appreciable duration (ERDHEIM 1914), and in Lignac-Fanconi disease it may in addition be secondary to renal disease. Bone changes suggestive of hyperparathyroidism are not conspicuous in Lignac-Fanconi disease, certainly less so than in classical hyperphosphataemic renal rickets. But even in the latter the changes are mainly and essentially rachitic. Our experience is the same as that of FOLLIS Jr. (1950), who studied the bone pathology in 16 cases of renal disease and found rachitic changes considerably more frequently than osteitis fibrosa.

Remissions and relapses of the rachitic process in Lignac-Fanconi disease are recognisable in some cases by the duplication or multiplication of zones of provisional calcification.

Parathyroids : Only a few reports on parathyroids in Lignac-Fanconi disease are available in the literature. Of the 28 cases listed in Table 2 in only eight is reference made to parathyroids. Enlargement of one or more parathyroids was found by BENOIT (1935), RUSSELL and BARRIE, Case 1 (1936), RÖSSLE (1938), and SCHÜMMELFEDER (1952), while STURZENEGGER (1939) describes them as pinhead-sized, LOOSER (1944) as macroscopically not clearly recognisable, and ROULET (1941) and DRABLØS (1951) as normal. The macroscopical identification of parathyroids in childhood is difficult not only because of their small size but also because the distinctive yellowish colour is often absent. We have, therefore, relied on measurements at autopsy only in Case 9 ; while in Case 15 the left half and in Case 16 the whole organs of the neck were cut in serial sections and measurements made microscopically on the sections. In Case 9 the parathyroids were normal in size but histologically there was a structure more compact than normal and there was a nuclear hyperchromasia present ; the predominant cell was a cloudy chief cell. In Cases 15 and 16 the parathyroids were markedly enlarged and the predominant cell was a water-clear chief cell. Our three cases and those of RUSSELL and BARRIE (1936) and of ROULET (1941) had advanced glomerular changes, while in the kidneys reported by BENOIT (1935), by RÖSSLE (1938) and by SCHÜMMELFEDER (1952) none or only mild glomerular changes

were found. The number of cases studied is too small for a definite statement, but it appears that though higher degrees of hypertrophy are due to renal disease some parathyroid hypertrophy occurs before renal failure ; it is probably secondary to rickets, as in ordinary vitamin-deficiency rickets (ERDHEIM 1914).

Except for the parathyroids the endocrine glands show no significant changes. The bilateral suprarenal necrosis in Case 9 was the result of a complicating infection, and the ciliated epithelial cyst of the pituitary in Case 16 was certainly a coincidental developmental abnormality.

Pathogenesis

Several hypotheses have been put forward with regard to the pathogenesis of Lignac-Fanconi disease, varying according to the approach of the author as a clinician or a pathologist. In many instances little or no significance was attached to the question of how far the Fanconi syndrome was identical with cystine storage disease.

(1) Lignac (1924, 1926) assumed as a matter of fact that the deposition of cystine crystals in a variety of organs was the result of a metabolic disorder and that this disorder was identical with that in cystinuria with cystine lithiasis (cystine-lysinuria), an assumption suggested by the observation of urinary cystine calculi in one of his cases. Russell and Barrie (1936) likewise assumed a defect in the cystine metabolism and considered the deposition of cystine crystals in the cells of the reticulo-endothelial system as "a complication of cystinuria." Among recent authors dealing with this problem the main advocate of a metabolic error identical with that in cystine-lysinuria is Freudenberg (1949). Referring to the work of Brand and his associates (1935) Freudenberg gave the following diagram to illustrate the metabolic pathway of methionine :

methionine——> homocysteine——> cystathionine——>
cysteine ——> sulphate

↓

cystine

In cystine storage disease and cystine-lysinuria he postulated the failure of oxidative degradation of cysteine to sulphate, resulting in its increased oxidation to cystine. He considered that cystine storage was due to inefficient elimination of increased quantities of cystine and was to be expected in infants and young children, as "cystinuria is impossible in young children, with their functionally immature kidneys."

(2) A second theory was conceived by Fanconi (1936), who assumed an inborn functional defect of the proximal tubules, consisting of inability to reabsorb glucose, phosphate and certain cations from the glomerular filtrate. With the discovery of aminoaciduria Fanconi (1942, 1946) changed his opinion in favour of a general metabolic disorder of aminoacids and amines and suggested for the disease the name " diabetes aminicus et acidaminicus." McCune and his associates (1943) extended Fanconi's original renal theory to explain the aminoaciduria which they attributed to defective tubular resorption. They suggested that cystine storage might develop as the result of prolonged renal waste of metabolites essential to cystine metabolism ; this might lead to disturbed oxidation of cystine and its consequent deposition in the tissues. Alternatively, cases with cystine storage represent an etiologically independent entity in which the deposit of cystine in the kidney leads to glomerular and tubular changes, thereby producing the chain of consequences which characterise cases of Fanconi's syndrome without cystine storage. In recent years the renal theory of Lignac-Fanconi disease gained much ground as a result of the classical studies of Dent (1947, 1952) and of Stowers and Dent (1947). Fanconi himself (1950, 1951) reverted to the renal hypothesis, which explained the aminoaciduria, glycosuria, increased urinary loss of bases and presumptive hyperphosphaturia all as the result of a congenital defect in the reabsorptive capacity of the proximal tubules.

(3) Fanconi's second theory (1946) mentioned under (2) assumed a general disturbance of the deamination of many aminoacids.

(4) Linnewch (1951) suggested a defect in the utilisation of aminoacids in the kidney in the case of both cystinuria and cystinosis and connected the difference between the two disorders not with age or the extent of kidney damage but with the quantity of unutilised cystine. Cystine storage occurs when the metabolism of cystine in the kidney is so severely disturbed as to cause insufficient excretion.

(5) Baar (1950) and Bickel (1950) suggested as the primary disturbance a defect in the intracellular metabolism of aminoacids within the reticuloendothelial system with the exception of the littoral cells.

Before giving a more detailed account of our own pathogenetical considerations there follows a critical discussion of theories (1)–(4).

Theory 1 : The assumption of a congenital disturbance in a single phase of the chain of metabolic processes concerning cystine is attractive and would place Lignac-Fanconi disease and cystine-lysinuria on a

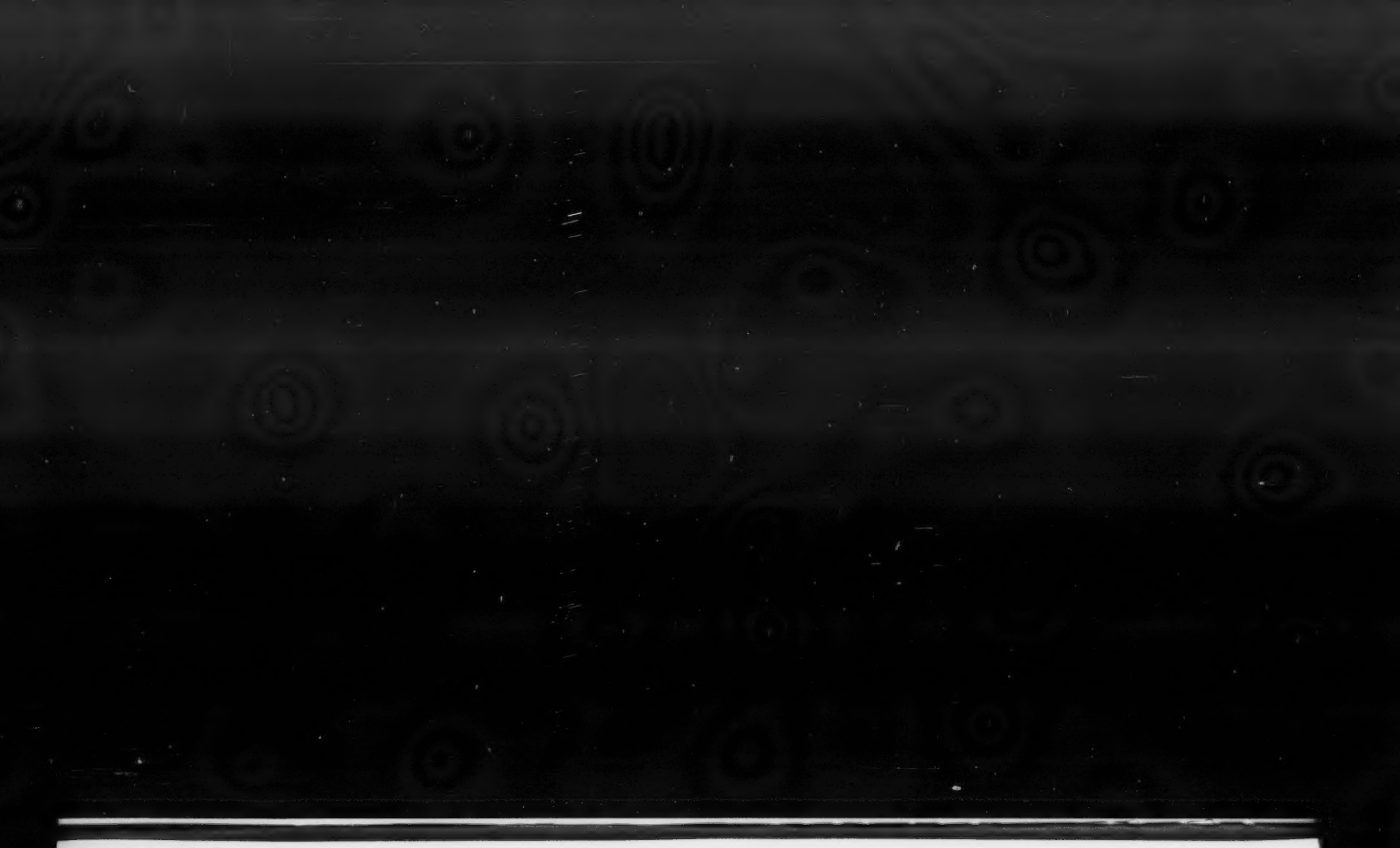


Table 2. CASES OF LIGNAC-FANCONI DISEASE SO

	Author		Cystine storage proved p.m.	Cystine storage proved in vivo	Detailed Histology	
1	Abderhalden 1903 Kaufmann 1922		+	—	Incomplete	
2	Lignac 1924	Case 1	+	—	Yes	Albuminous
3		Case 2	+	—	Yes	Kidney pal
4	1926	Case 3	+	—	Yes	Kidney pale parenchyma
5	Benoit 1935		+	—	Yes	Pyelonephrit epitheli
6	Fanconi 1936	Case 1	?	—	Yes Sturzenegger 1939	Suspicious severe t
7		Case 3	+	Cystinuria	Yes Looser 1944	Glomerulo-s
8	Russell and Barrie, 1936	Case 1	+	—	Yes	
9		Case 2	+	—	Yes	Complete
10	Beumer and Wepler 1937		+	—	Yes	Glomeruli well preser
11	Rössle 1938		+	—	Yes	Glomeruli i t
12	Pache 1940	Case 1		Cystinuria, sister of Case 2 and of Linneweh's case.	Died. No p.m.	
13		Case 2		Brother of Case 1 and of Linneweh's case.	P.M. mentioned by Freu	
14	Hottinger 1941		+	In eyes and bone-marrow +	Yes Roulet 1941	Kidney atrop
15	Fanconi 1946	Case 3	+	Bone-marrow +	Yes Waser 1946	"Glomerulon
16	Hottinger 1947		+	—	Yes, but rather short	
17	Ullrich 1948			In eyes by slit-lamp +, crystals disappeared in biopsy (formalin).	Died. No p.m.	
18	Fanconi and Bickel 1949	Case 2		In bone-marrow +, sister of Monod's case.	Died. No p.m.	
19	D'Avignon and Vahlquist 1949			In eyes by slit-lamp and biopsy +. Bone-marrow : no crystals found.	Alive	
20	Clarke and	Case 1	+	—	Very short meeting re	
21	Jackson 1950	Case 2	+			
22	Drabløs 1951		+	In eyes by slit-lamp and biopsy +. Bone-marrow : no crystals found.	Yes, brief preliminary report	
23	Linneweh 1951	Case 1	+	In eyes by slit-lamp +. Bone-marrow +. Brother of Pache's cases.	Died, p.m., but no detailed report	
24	King and Lochridge 1951		+	—	Yes	Necrosis of tu u
25	Monod 1951			In eyes by slit-lamp +. Bone-marrow +. Brother of Case 2, Fanconi and Bickel.	Died. No p.m.	
26	Schümmelfeder 1952		+	—	Yes	Mild glomer
27	Dimson 1952	Case 1		In eyes by slit-lamp +.	Alive	
28		Case 2		In eyes by slit-lamp +.	Alive	

Cases being prepared for publication or unpublished (Cystine storage proved).

14 additional cases in this series, 2 of which will be published in detail by Dr. R. J. K. Brown, 1 by Dr. Boehncke *et al.*
1 unpublished case in St. Mary's Hospital, Portsmouth (Drs. R. D. Clay, E. M. Darmady and M. Hawkins).
1 unpublished case in the Children's Hospital, Birmingham (Prof. J. M. Smellie).
1 unpublished case in the Children's Hospital, Cardiff (Dr. J. Jacobs).
1 unpublished case in the Children's Hospital, Hamburg (Prof. Schaefer).
1 unpublished case in the Children's Hospital, Bristol (Dr. B. Corner, Prof. T. F. Hewer).
5 unpublished cases in the Children's Hospital, Great Ormond Street, London (three cases of Dr. W. G. Wyllie, one case
1 unpublished case in the Univ. College Hospital, London (Dr. C. E. Dent).
1 unpublished case in the Children's Hospital, Giessen (Prof. Hungerland).

Total · 54 **proved cases of Lignac-Fanconi disease.**

SE SO FAR OBSERVED (with notes on histological findings).

Kidney Histology	Bone Histology	Parathyroids
No report	No report	Not mentioned
buminous degeneration of proximal and distal convoluted tubules.	No detailed report	No report
dney pale, swollen, cortex grey. Acute, possibly pyelogenic nephritis	No detailed report	No report
dney pale, swollen, cortex grey, cloudy swelling of renchyma, non-purulent pyelonephritis. Cystine stones	No detailed report	Not mentioned
elonephritic changes, proliferation of glomerular epithelium. No mention of tubular changes	Rickets	Enlarged but normal cellular structure.
spicious histological changes. Normal glomeruli, severe tubular changes, slight interstitial nephritis	Very detailed, "renal and avitaminotic rickets".	"As big as a pin head".
omerulo-sclerotic kidney atrophy, chronic interstitial nephritis, tubulo-nephrosis	Detailed histology. Severe active rickets, pronounced marrow fibrosis, dissecting bone resorption	"Not clearly recognizable, though microscopically some parathyroid tissue traceable. Normal tissue".
Advanced Bright's disease.	Typical severe rickets	Cystine deposit. "Enlargement of parathyroid glands".
mplete glomerular destruction, tubular atrophy. (Chronic Bright's disease).	Not examined	Not mentioned
omeruli underdeveloped, cubic epithelium relatively l preserved. Tubular atrophy, proliferation of interstitial tissue.	No	Not mentioned
omeruli intact, tubuli partly atrophic, partly hypertrophic. Severe tubular nephrosis.	Cartilage shaft junctions of ribs normal.	Left inferior parathyroid body enlarged, normal cellular structure.
by Freudenberg 1949, but no description		
dney atrophy, glomerular sclerosis, interstitial fibrosis, tubular atrophy.	Rickets and osteosclerosis, detailed description.	Not enlarged
lomerulonephrosis," tubular degeneration, interstitial infiltrates.	Mild changes, suggestive of healed rickets.	Not mentioned
No kidney changes.	"Active rickets, broad ostoid seams".	Not mentioned
eeting report of two cases of cystinosis.		
No kidney changes.	Not mentioned	Normal
No detailed description	No detailed description	Not mentioned
rosis of tubules. Glomeruli normal. Marked megaloureter, congenital urethral valve.	No bone description	Not mentioned
d glomerular changes, tubular degeneration, interstitial infiltrates.	Rickets	Enlarged

cke *et al.* (Cases 3 and 12 are included above).
ns).

, one case each of Prof. A. Moncrieff and Dr. P. R. Evans).

par with inborn errors such as alkaptonuria and phenylketonuria. There are, however, several objections to this theory.

(a) The aminoacid pattern in the urine of Lignac-Fanconi disease and of cystine-lysinuria are entirely different (see Part 1, page 19) and remain so over years of observation, probably throughout life. Transitions from one pattern to the other have not been observed. Cystine excretion may be increased in both diseases but is unspecific, as it occurs in a variety of conditions (Part 1, page 16).

(b) In their clinical manifestations again, there is no similarity or transition between cystine-lysinuria and Lignac-Fanconi disease, except for the observation of a cystine stone in one case of Lignac-Fanconi disease (Lignac 1924). Cystine stone formation might, however, be expected occasionally in any condition with an increased urinary excretion of cystine. Freudenberg's hypothesis is further contradicted by the fact that cystinuria and cystine-lysinuria are not uncommon in infancy and childhood (Part 1, page 20).

(c) The occurrence of cystine-lysinuria and Lignac-Fanconi disease in the same family is exceptional. Lewis (1932) in serial investigations of a healthy student population found 1 in 600 to be cystinuric. This may account for its occasional occurrence in relatives of patients with Lignac-Fanconi disease, or there may be some genetic link between two otherwise different diseases.

(d) The general aminoaciduria of Lignac-Fanconi disease is not explained by theory (1). In unpublished experiments on young rats extending over a period of five months we were unable to produce general aminoaciduria by daily injections of saturated solution of cystine. In man massive cystine excretion in cystine-lysinuria does not lead to general aminoaciduria.

(e) Cystine storage cannot be due to progressive glomerular destruction, as it has been found in the first year of life (Hottinger 1947) without glomerular changes or an excessive rise in the cystine blood level (Part 7, Table 5) and with considerable cystine excretion in the urine.

(f) As shown in the discussion of histopathological findings, cystine is not taken up by cells of the reticulo-endothelial system from oversaturated body fluids, but there is convincing evidence that it crystallises at the site of excessive formation within the cells. The localisation of crystals cannot be explained by phagocytosis.

We conclude that Lignac-Fanconi disease and cystine-lysinuria are two different disorders. Lignac-Fanconi disease cannot be explained on the assumption of an isolated disturbance in the cystine

metabolism, and cystine storage is not the result of insufficient kidney clearance with consequent phagocytosis.

Theory 2 : The renal theory is mainly based on the assumption of low or at least normal aminoacid blood levels, of hyperphosphaturia and renal glycosuria.

(a) The blood level of various aminoacids such as valine, the leucines, phenylalanine, tyrosine and glutamic acid has been shown to be raised (see Part 7). As regards the cystine storage Dent himself (1952) has recently admitted that this cannot be explained by any theory involving the renal tubule. A compensatory decrease in tubular reabsorption of aminoacids, however, may possibly be an additional factor and, together with polyuria, will tend to keep the blood levels only slightly raised in some aminoacids and not significantly at all in others. A decreased tubular reabsorption of aminoacids need not be a pathological process at all, as Pitts (1944) has shown that reabsorption of glycine, dl-alanine, l-glutamic acid and l-arginine decreases rapidly in normal dogs as the load is increased.

(b) Hyperphosphaturia is either absent or only relative. The balance studies described in Part 7 show that urinary phosphate excretion, expressed as a percentage of intake or of total excretion, was reduced in most cases. Hypophosphataemia is, therefore, not the result of urinary loss of phosphate. If, however, the phosphaturia is considered in relation to blood levels, an increased phosphate clearance is likely in some cases with pronounced hypophosphataemia, though actual clearance data are still lacking. According to Milne, Stanbury and Thomson (1952) in normal adults the phosphate clearance is zero at plasma levels below 2.2 mg. per 100 ml. A urinary phosphate excretion which is normal in relation to the intake but increased for the given blood level has been called relative hyperphosphaturia and has been demonstrated in ordinary infantile rickets (Schabad 1910, György 1929), in nutritional deficiency in China (Miles and Feng 1925, Liu, Hannon, Chu, Chen, Chou and Wang 1935) and in steatorrhoea (Bauer and Marble 1932, Milne 1951). It is probably the result of parathyroid hyperactivity, which depresses phosphate reabsorption (Albright 1936) and which is commonly found in rickets and osteomalacia (Erdheim 1914). It has been mentioned above that parathyroid hyperplasia is probably more common in Lignac-Fanconi disease than it would appear from the literature. In the only two cases in which we have determined the size of parathyroids by means of serial sections the enlargement was marked. An additional factor leading to "relative hyperphosphaturia" may be the aminoaciduria *per se* since

Ayer, Schiess and Pitts (1947) showed that infusion of alanine and glycine may depress phosphate reabsorption to about 35 per cent of the control value.

(c) "Renal glycosuria" is also no proof of the primary nature of the kidney lesion. The glycosuria may be due to glucose formation from aminoacids in the kidneys (see Part 3, page 62) or to tubular damage in the course of the disease. The secondary nature of the development of glycosuria is suggested by the fact that it is usually much less pronounced than the aminoaciduria and may be minimal or completely absent. In this connection recent rat experiments (unpublished) over

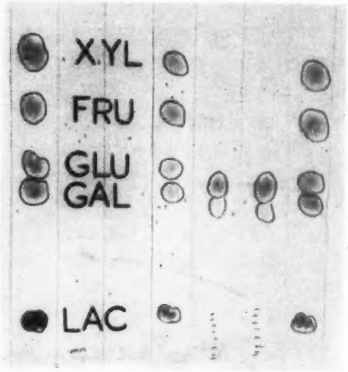


Fig. 32. Sugar chromatogram with urine of untreated control rats (runs No. 2, 3), and of rats treated with cystine (runs No. 5, 6). No. 1, 4 and 7 show runs with pure sugar solutions (LAC, lactose, GAL, galactose, GLU, glucose, FRU, fructose, XYL, xylose).

periods of up to five months were of interest. Animals injected daily intramuscularly with cystine or with casein hydrolysate excreted significantly more glucose than the control animals, as shown by paper chromatography (Fig. 32). There was no histological evidence of tubular damage nor any definite decrease in phosphatase activity or alteration of its distribution.

In conclusion, the assumption of a tubular dysfunction as the primary lesion in Lignac-Fanconi disease is not substantiated by evidence. This theory cannot explain the aminoacidaemia and cystine storage, nor are hypophosphataemia, hypocalcaemia and bone decalcification due to loss of phosphorus and calcium through the

kidney. Renal glycosuria may be a secondary phenomenon resulting from aminoaciduria.

Theory 4 : The objections to theory 1 may also be sustained against Linneweh's hypothesis. His suggestion that the excess of cystine originates in the kidneys and is secondarily taken up by the reticuloendothelial system seems to us to be disproved by our histopathological studies.

Theory 3 and 5 : Having ruled out a renal lesion as the origin of Lignac-Fanconi disease we are confronted with a metabolic disorder as the only remaining possibility. Such a disorder might be secondary to an endocrine disease, but no constant changes in the endocrine glands have been found in Lignac-Fanconi disease, with the possible exception of the parathyroids. We therefore suggest that a congenital disturbance of the enzyme system concerned with protein metabolism is at the root of the disease. Our hypothetical conception of the pathogenesis is as follows :

The visible manifestation of the disturbance of aminoacid metabolism is cystine storage. We have presented evidence that cystine crystallises intracellularly at the site of its excessive accumulation, namely in the cells of the reticuloendothelial system with exception of the littoral cells. In this Lignac-Fanconi disease is comparable to Gaucher's and Niemann-Pick's diseases, which according to Tannhauser's (1940) attractive hypothesis are disorders of the intracellular lipid metabolism.

We have tried to demonstrate an increase of other aminoacids besides cystine in the tissues by histochemical methods. Unfortunately these methods are not very satisfactory and, except for the diffuse and strongly positive ninhydrin reaction in sections from a biopsy specimen of conjunctiva, we obtained clear results only with Thomas' method for arginine. Of four livers from Lignac-Fanconi disease three showed a definitely stronger colour reaction than the controls. The interpretation of this result is, however, equivocal, since the reaction does not permit a differentiation between arginine, arginine polypeptides and proteins rich in arginine.

General aminoaciduria and aminoacidaemia suggest that the metabolic disturbance is not limited to cystine but extends to the whole aminoacid metabolism. The general aminoaciduria does not appear to be caused by the toxic action of cystine (see above). The site of the disturbance is probably the reticuloendothelial system, as evidenced by intracellular cystine accumulation. The special rôle of this cell system in the synthesis of proteins was indicated by Müller (1905), Jürgens and Gebhardt (1934), Jürgens (1938), Heinlein (1943) and Ewerbeck (1952).

We have no evidence as to the nature of the aminoacid disturbance. Fanconi's theory 3 suggested a general defect in the deamination of

aminoacids but our studies lend no support to this idea, as the urea formation, and in some cases the ammonia production as well, are normal or even excessive. After addition of casein hydrolysate to the food the ratio amino nitrogen to total nitrogen remained unchanged. It seems to us more likely that the anabolic phase of protein metabolism is affected. This disturbance may only be quantitative and consist in a slowing-down of protein synthesis. An anabolic block concerning one or only a few aminoacids is unlikely, as hydrolysate of plasma protein from patients with Lignac-Fanconi disease gives the same aminoacid pattern as that of normal individuals (Part 3, Fig. 6). The aminoacid pattern in the urine of our patients, in contrast to any other aminoaciduria we have investigated, shows a striking similarity to the aminoacid pattern of hydrolysed plasma protein and of protein-free plasma filtrate of the patient and also of normal subjects (Part 3, Fig. 10). It is thus reasonable to assume that all protein-building aminoacids are involved in the disturbance. Owing to the partial failure of the body to utilise them for the synthesis of proteins they accumulate in the body fluids and overflow into the urine. Such a disturbance would account satisfactorily for the dwarfing, which cannot be due solely to rickets or renal disease, as both are later complications of the disease and dwarfing can precede them.

Assuming a merely quantitative disturbance of protein synthesis, the electrophoretic pattern of serum protein need likewise not necessarily be abnormal (Linneweh 1951), though in some cases it is definitely pathological. In addition to those mentioned in Part 3, p. 45, and in Part 6, p. 122, we reproduce the curve obtained by filter paper electrophoresis with spectrophotometric analysis in Case 8, together with four curves from normal children (Fig. 33). An increase in α-globulins with comparatively low γ-globulins is evident. It is of interest in this connection that Cohen and his associates (1947) found in a case of acute reticulosis (Letterer-Siwe's disease) an increase of glubulins, particularly of the α fraction.

The relationship between the metabolic disorder of Lignac-Fanconi disease and its renal pathology is in many respects obscure. There are, however, a few well-established facts. Cystine storage may be present without or with only trivial changes in the kidneys. The renal pathology is clearly progressive with interstitial and tubulo-degenerative changes with or without glomerulonephrosis and focal glomerulonecrosis progressing to diffuse fibrosis, glomerulosclerosis, tubular atrophy and cystic dilatation. It follows that the renal changes are not congenital developmental in origin. The modified tubular epithelium is an acquired property and the "pseudocolloid cysts" the result of distal obstruction comparable to that in chronic pyelonephritis.

The toxic effect of cystine upon the kidneys has been repeatedly assumed and has been substantiated by animal experiments (for literature see Freudenberg, 1949). Changes comparable with those in Lignac-Fanconi disease have, however, never been obtained and in our own experiments intramuscular injections of cystine or of casein hydrolysate did not result in any significant renal changes, though young animals were used. Chronic cystine-lysinuria may exist in early childhood and last for many years without ever leading to renal

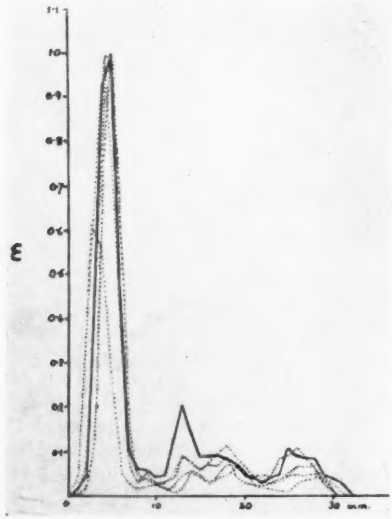


Fig. 33. Electrophoretic curves of serum from a case of Lignac-Fanconi disease (solid line), and from 4 normal children (dotted lines). Filter paper electrophoresis and Unicam spectrophotometer.

symptoms, though the cystine excretion is usually far greater than that in Lignac-Fanconi disease. Another chronic and massive amino-aciduria, phenylalaninuria of phenylpyruvic oligophrenia, may last a lifetime without leading to kidney disease. We are, therefore, not yet satisfied that the kidney lesion in Lignac-Fanconi disease is due to the toxic effect of cystine or any other aminoacid. At the present time the relationship between the metabolic disorder and the renal changes which are present in all but a few of the cases can only be generally

described as "chronic interstitial nephritis of disorders of protein metabolism."

Under this heading Zollinger (1945) describes a group of kidney diseases, which include multiple myeloma, lipoid nephrosis, amyloidosis, gout and also one example of cystine storage disease. Though the histological appearance of kidneys varies in these diseases, they have in common the association with some disturbance of protein metabolism. That endogenous degradation products of protein may cause renal changes is supported by several observations (Spühler and Zollinger 1943, Zollinger 1945). The kidney damage in haemoglobinuric lower nephron nephrosis and in crush syndrome is probably due rather to the toxic action of these degradation products than to the blockage of tubules by haemoglobin or myoglobin casts. It is conceivable that a disturbance in the synthesis of body protein may lead to the accumulation of similar products, and in this connection the constant and considerable increase of organic acids in the urine and probably also in the blood of our patients should be borne in mind.

The origin of the rickets and acidosis is also still speculative. The problem is not rendered simpler by the fact that the rickets is not "renal" but due to poor intestinal absorption. The finding of phosphatase depletion in various organs may be of significance, as it suggests a defect in enzyme systems other than those responsible for protein anabolism. Unfortunately the intestinal wall, where a phosphatase deficiency might well have been expected, does not seem to be affected.

The acidosis has been shown to be due to a variety of factors, renal as well as extrarenal (Parts 3 and 7). We have already suggested that the excess of organic acids in the urine may originate in the disturbed protein metabolism. Moreover, the severity of the acidosis in young and acutely ill patients with strong aminoaciduria and other signs of a grave metabolic disturbance indicates a primarily metabolic origin, for it may be mild or not demonstrable at all in the chronic cases despite advanced kidney damage (Case 8). In Lignac-Fanconi disease there is no evidence for the suggestion by Albright and Reifenstein (1948) that the acidosis may lead to rickets by directing the base calcium into the urine. Calciuria was not a feature of our cases nor was there any correlation between the severity of the acidosis and of the rickets. This was clearly demonstrated in a recent case, a patient of Professor Smellie, who suffered from Lignac-Fanconi disease with severe rickets despite a normal CO_2—combining power of the plasma. (This was never below 23 mEq./l and was usually above 27 mEq./l).

To sum up, our view is that the complex disease described in the eight parts of this publication originates in a primary congenital disorder of protein anabolism. On this primary disorder we are inclined to put the blame for the principal features of the disease, such

as dwarfing, aminoaciduria, cystine storage, the renal lesion, acidosis and rickets, though we realise only too well how far we are from understanding the interplay of these factors. Further investigations (including animal experiments, clearance and tracer work) are planned and much work must be done before substantial factual evidence can be adduced to prove or disprove our hypothesis.

Summary

Detailed gross anatomical and histopathological findings in six cases of Lignac-Fanconi disease are described and discussed. An attempt is made to present the natural history of individual organ changes, particularly those of the kidney. It is interesting to note that the disease in its early stages can occur without kidney changes. The bone changes are essentially rachitic ; there is no feature of the histopathology of the bones which is not met with in rickets, particularly in "rachitis tarda." Bone changes suggestive of hyperparathyroidism are not conspicuous in this disease. The parathyroids were markedly hypertrophic in two of our cases ; hypertrophy in other cases cannot be excluded.

The phosphatase reaction was negative in all kidneys examined, reduced in liver, spleen and bone-marrow but normal in the small intestine. Negative phosphatase reaction in the kidney has also been found in chronic inflammatory kidney disease and is not specific for Lignac-Fanconi disease.

Methods for demonstration and identification of cystine crystals in tissue sections, bone-marrow films and lymph node imprints are described, together with experiments which account for the difference between the crystallographic appearance of cystine in tissues and cystine crystallised from aqueous solutions. In our opinion a lymph node biopsy is the most reliable procedure for a definite diagnosis of Lignac-Fanconi disease *in vivo*, though bone-marrow sections and/or films are usually sufficient for the purpose.

The intracellular localisation of cystine crystals is stressed, and histopathological and experimental evidence is given for the assumption that cystine storage is not due to phagocytosis, but that cystine crystallises at the site of its formation, namely in a certain part of the reticuloendothelial system.

A critical review is given of various hypotheses concerning the pathogenesis of Lignac-Fanconi disease. The hypothesis is put forward that Lignac-Fanconi disease is a disorder of protein metabolism, probably of its anabolic phase. As a disorder of intracellular metabolism, the disease is likened to true lipidoses.

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(LIGNAC–FANCONI DISEASE)

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ELECTROCARDIOGRAPHIC STUDIES IN SCARLET FEVER

AN INVESTIGATION WITH SPECIAL REFERENCE TO THE EFFECT OF PENICILLIN TREATMENT

BY

MAJ LEVANDER-LINDGREN

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ELECTROCARDIOGRAPHIC STUDIES IN SCARLET FEVER

An investigation with special reference to the effect of penicillin treatment

By

MAJ LEVANDER-LINDGREN

STOCKHOLM 1952

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STOCKHOLM 1952

To my Mother

and the memory of

my Father

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Introduction

Our interests in myocarditis, as in many other subjects, has changed through the years. Periods of over-diagnosis have caused a loss of confidence and led to periods in which the diagnosis was ignored or neglected. In 1941 when O. Saphir and I. Gore began their series of articles on myocarditis based on studies of postmortem sections, we were in a period when myocarditis was too seldom taken into consideration. By systematic studies of the pathological anatomy of the myocardium they showed how infectious diseases of various etiologies (bacterial, viral, Rickettsial and mycotic) might be associated with myocarditis. A clinical diagnosis of myocarditis had been made in only one third of the cases which these authors saw at autopsy although many of the overlooked cases had had clinical evidence of heart involvement. However, during recent years the clinicians have paid more and more attention to the heart in cases of infectious disease. A number of articles have shown that our ordinary infectious diseases, even the common cold, are followed by an unexpectedly high incidence of myocarditis. It is apparently, however, frequently subclinical and symptoms are often absent at rest. The diagnose is then made only by electrocardiography and associated procedures. It is important not to miss the diagnosis, however, because some of these cases, when questioned more carefully, are found to have had cardiac symptoms for some time during convalescense even after the alterations in the electrocardiogram have regressed.

The more chemotherapy and the antibiotics shorten the course of the illness — and the pace of the present times demands the earliest possible return to work — the more important is a correct diagnosis of myocarditis as a complication. We must also determine the extent to which the antibiotics influence the incidence of myocarditis. In addition to the practical considerations, the following question, of theoretical interest, also arises: Are the shortening of the illness and the bacteriological purge which treatment with penicillin and the other antibiotics accomplishes, sufficient to prevent the occurrence of myocarditis? In other cases does such treatment have any effect at all, as, for example, on the severity of the myocarditis?

A number of articles have also been published about the penicillin treatment of scarlatina. (For references see bibliography.) These have, however, been mostly with short series of patients. The marked effect of penicillin on the

bacterial complications, and the associated impairment of immunity with frequent relapses, are widely confirmed. Several studies of myocarditis, which is a toxic complication, were published before penicillin came into use, but it has not been studied closely in penicillin-treated scarlatina. It cannot be expected that the incidence of myocarditis will be constant, since the character of scarlatina varies geographically as well as from time to time in the same locality. Jersild, in Copenhagen, who has made the most extensive studies of penicillin-treated scarlatina, reports an incidence of 0.5 per cent myocarditis in a group of 200 cases treated with penicillin and the same frequency in a simultaneous series of 200 patients treated with sulfa. In his investigation myocarditis was the only complication which was not lower in incidence in the penicillin-treated group. He has the same experience regarding the frequency of myocarditis in the rest of his large penicillin series. Nor have Lasch (1950) and Paetzold (1950) found any effect of penicillin therapy on the myocarditis of scarlet fever. It seems that more detailed analysis of the effect from penicillin on the frequency of myocarditis has not previously been given, and such an analysis demands parallell series of non-penicillin treated controls. However, myocarditis, as well as nephritis, is the complication which must determine the length of treatment and the subsequent period of observation.

Large parallell series of control cases and cases treated with penicillin were admitted to this hospital as reported by Ström (1948—1950). Regular clinical examinations and electrocardiograms have been made on these patients for a considerable time after the beginning of the illness. We have thus obtained valuable material for a scientific analysis of the effect of penicillin on the myocarditis which has been the main purpose of this work. During the last year of these investigations the control series could not be completed for practical reasons. However, patients from this period as well as desquamating cases showed interesting electrocardiographical changes. For this reason these cases will also be described in the present study.

A big effort has been made to differentiate between myocarditis and such changes in the electrocardiogram as may be caused by changes in autonomic tone, extreme constitutional variations, old lesions or congenital anomalies. Therefore, during the course of investigation the need for both supplementary methods of study and prolonged periods of observation has been gradually appreciated.

In addition to myocarditis, an account has been given of electrocardiographical changes not considered due to scarlatinal myocarditis. They are of interest both from the standpoint of differential diagnosis and as a finding in apparently healthy individuals. It is hoped that the experience gained from this material may also be of value in electrocardiographic studies in other diseases. By means of follow-up studies the author also attempted to establish the prognosis in scarlatina myocarditis for the first few years after infection.

CHAPTER 1

The Material

The patients are those cared for in the Stockholm Epidemic Hospital during the 3 year period from September 1946 through June 1949. The epidemics were of a relatively mild variety with only occasional severe cases.

Distribution by Groups

The dosage and route of administration of penicillin was different in each of the three year periods. The material has therefore been classified in groups. The cases treated with penicillin are indicated by the letter P, with various sub-indices: the simultaneous control cases, by the letter C, with appropriate sub-indices. Patients admitted without bacterial complications were assigned to the various groups in the order of admission without any special selection. The isolated severe cases received convalescent serum. When an incidental complication made it necessary, penicillin therapy was of course instituted even in the control cases. When other contagious diseases, independent of scarlatina, developed during the course of observation, the patients affected were excluded from the series.

The First Period of Treatment (P_1 and C_1)

This period extended from September 1946 through August 1947. In the penicillin group P_1, there were 228 patients who received 8 intramuscular injections per day for 6 days. After 6 months the dosage was increased from 10.000 Oxford Units (O. U.) to 20.000 O. U. for adults and correspondingly smaller doses for children (see table below). Schenley's penicillin has been used throughout the entire period.

Daily Dosage of Penicillin in O.U.)*

Age	1/9/1946—17/3/1947	18/3/1947—31/8/1947
Infants	1500×8	3000×8
1—4 years	2500×8	5000×8
5—9 years	5000×8	10000×8
10—14 years	7500×8	15000×8
> 14 years	10000×8	20000×8

*) The first dose is double that of subsequent doses.

When the patients had had 3 consecutive negative nose-throat cultures for hemolytic streptococcus they were transferred to a ward with rooms of a capacity of up to 14.

The control cases, C_1, numbering 221 patients, were kept for one week in smaller rooms in the acute section separted from the penicillin cases. They were subsequently transferred to wards of the same size as were the penicillin cases.

The hospital confinement for both P_1 and C_1 uncomplicated cases was 4 weeks, where there were no children in the home, and in other cases 6 weeks. Electrocardiograms were recorded at least twice during the illness. For additional information on these groups see Ström (1948).

The Second Period of Treatment (P_2, P_5, and C_2)

The patients from this period, September 1947 through August 1948, have been assigned to three groups. In the first group, P_2, consisting of 222 patients, penicillin was begun on the second hospital day. In the second group, P_5, 211 patients, penicillin was begun on the fifth hospital day. The dosage was the same in both groups, and the penicillin was given by intramuscular injections for 6 days 3 times a day at first and later twice a day. (See table below.)

Daily Dosage of Penicillin in O.U.

Age	1/9/1947—6/2/1948	7/2/1948—31/8/1948
Infants	10000×3	20000×2
1—4 years	20000×3	40000×2
5—9 years	40000×3	60000×2
10—14 years	60000×3	80000×2
> 14 years	80000×3	100000×2

When the cultures for hemolytic streptococcus were negative for 3 concecutive days the patients in both groups P_2 and P_5 were transferred to the same convalescent wards. The third group, the controls, C_2, consisted of 219 patients all of whom were kept in isolation rooms.

All of these patients without complications were kept in the hospital for 3 weeks, and electrocardiograms were taken once a week. They were subsequently seen in the outpatient clinic at the end of 1 and 3 weeks following discharge from the hospital. At the beginning of these studies a third check-up at the end of 7 weeks was also carried out. This was omitted when it was discovered that no new complications were found. At the time of the follow-up a general examination was carried out, including auscultation of the heart, as well as a urinalysis, electrocardiography and a determination of the sedimentation rate, and the antistreptolysin titre. For further data concerning these patients see Ström (1949).

The Third Period of Treatment (P_{pk}, P_{po} and C_3)

During this period, including the patients from the first of September 1948 through the thirtieth May 1949,*) two schemes of treatment were followed, one with intramuscular procaine-penicillin and one with amorphous oral penicillin. Procaine-penicillin was given to 368 patients, P_{pk}, in single daily injections until cultures for hemolytic streptococcus were negative for 3 days. Dosage, however, was always continued for at least 6 days. Oral penicillin, P_{po}, was given to 339 patients and was administered in a 5 per cent glucose solution 3 hours after each meal (3 times per day) until negative cultures were obtained for 3 days. The minimum period of dosage was 6 days. In both treatment series the dosage was increased after half the period had elapsed.

Daily Dosage of Procaine-Penicillin (I.U.)

Age	10/9/1948—8/3/1949	9/3/1949—31/6/1949
Infants	50000	75000
1—4 years	75000	100000
5—9 years	100000	125000
10—14 years	150000	150000
> 14 years	150000	200000

Daily Dosage of Oral Penicillin (I.U.)

Age	10/9/1948—8/3/1949	9/3/1949—31/6/1949
Infants	20000×3	40000×3
1—4 years	50000×3	75000×3
5—9 years	80000×3	100000×3
10—14 years	100000×3	125000×3
> 14 years	100000×3	150000×3

After treatment the patients from both groups were transferred to the same ward. It was found impracticable to have a simultaneous untreated control group confined in isolation rooms throughout the period. Only a smaller number of patients, 99, were available for the control group, C_3. The period to discharge was the same as for the previous group, 3 weeks for uncomplicated cases. The patients have been seen for check-up in the outpatient clinic 1 week and again 3 weeks after discharge. Each week during the time in the hospital as well as at both follow-ups electrocardiograms were taken, i. e. at least 5 per patient. For additional data regarding these patients see Ström 1950.

Group 0 — Cases not included in any of the above groups

When complications were already present at the time of admission, or incubation made special isolation necessary, or when the diagnosis was in

*) Patients from June 1949 were later excluded from the series because of artefacts in the electrocardiograms due to a faulty apparatus.

question until a later date, the patients have not been assigned to any of the treatment series. From September 1947, however, these cases were also included in the study. There is a total of 306 patients who have been assigned to this group indicated by the letter 0. The group includes patients treated both with and without penicillin. The period to discharge, follow-ups, and taking of electrocardiograms were the same as in the other groups.

Group D — Scarlatina cases admitted in the desquamation stage.

Patients admitted as late as the stage of desquamation have been included in the study since the first of September 1947. They have been treated with penicillin only when they were carriers of hemolytic streptococcus or suffering from complications. Hospital confinement, follow-ups and the taking of electrocardiograms have been based on the calculated date of onset of the illness in order to correspond to the patients in the exanthema stage. Group D consists of 505 patients.

Group I — Scarlatina cases complicated by other infectious diseases

To this group we have allocated all cases, which, in addition to the scarlatina, have developed other contagious diseases. 113 cases belong to the group. All the more usual infectious fevers were encountered: measles, rubeola, varicella, herpes zoster, mumps, pertussis, infectious hepatitis and tuberculosis.

An outline of the different series, their size, and the distribution between adults and children is given in table 1.

TABLE 1.

Distribution of the Material.

Year	Series	No. of patients	Adults	Children
1946—47	P_1	228	52	176
	C_1	221	36	185
1947—48	P_2	222	33	189
	P_5	211	18	193
	C_2	219	28	191
1948—49	P_{pk}	368	18	350
	P_{po}	339	17	322
	C_3	99	2	97
	O	306	26	280
	D	505	43	462
	I	113	6	107
Total		2831	279	2552

Explanation: "P" indicates cases treated with penicillin: "C", control cases. "O" indicates scarlatina cases not included in the regular groups, "D" desquamating cases, and "I" cases complicated with other infectious diseases.

CHAPTER 2

Metods of Investigation

Apparatus and Leads

For practical epidemiological reasons it was necessary to take the routine electrocardiograms in the wards using a small portable apparatus capable of recording one lead at a time (Elema Junior — System Elmquist). The 3 standard extremity leads were used as a routine measure. It was only during the last 3 years of this study that it was possible by means of an improved apparatus (Elema Triplex) to record precordial leads and unipolar extremity leads in cases of special interest. Unipolar extremity leads have been used according to Goldberger's method and precordial leads according to Wilson's or with the right arm as the indifferent electrode. Wilson's points 2, 4 or apex, and point 7, corresponding with the posterior axillary line have been used and in certain cases points 1, 3 and 6. Lead J is taken between point 7 or 6 and apex or point 4.

The apparatuses are of the amplifying type. The time recording is the same in all curves. The larger divisions correspond with 0.1 second and the smaller ones with 0.02 second. The apparatus is routinely calibrated so that 1 mV = 10 mm. Some films show horizontal lines.

The Frequency of the Electrocardiographic Examinations

As reported in the description of the different series, in most of them 5 or 6 routine electrocardiograms were taken for each patient using the ordinary extremity leads. Whenever pathological or suspicious electrocardiograms were recorded additional controls were made. In a smaller number of patients (208) electrocardiograms were taken more frequently during the period of hospitalization. The original intention was to make daily studies, but for practical reasons this was seldom possible. In each of these patients, however, at least 9 studies were made in the course of 3 weeks.

Follow-up Examinations

In addition to the routine follow-ups, the patients who had myocarditis or other changes in the electrocardiogram were called to the hospital for check-ups 1—4 years later. By this means an effort has been made to determine the

extent to which the myocarditis has caused subjective symptoms and whether the electrocardiogram, often both the resting and the "work" electrocardiogram, has become normal. In patients with doubtful pathological changes we have seen whether these remained and in other cases if they could be reproduced in response to changes in the physiological state. Attention has also been given to the development of cardiac murmurs with reference to a possible endocarditis. A total of 275 patients have appeared for this study.

The Function Test

The function test has frequently been of value as a supplement to the other studies. It has often been carried out before a patient who has had myocarditis was permitted to begin working again, in cases with questionable electrocardiographic changes, and in cases where alterations have remained constant over a long period of time. It is also valuable in the presence of subjective heart complaints when objective findings are laeking in the usual examination.

We have carried out the function test according to Sjöstrand's (1947), and Wahlunds (1945 and 1948) method. After half an hour's rest the pulse and respiratory frequencies are determined. The patient then pedals on a stationary bicycle ergometer with a revolution counter and an adjustable oil pump resistance. The load is gradually increased and adapted to the individual (200, 300, 450, 600, 900, 1200 Kg.M./min.). Each load is maintained for 6 minutes often with several consecutive periods at increasing loads. The pulse and respiratory frequencies are taken after the second, fourth, and sixth minutes. According to Wahlund the oxygen consumption is fairly uniform for various individuals at the same work load on the bicycle ergometer. The individual's functional capacity is determined by the heaviest work under which he is capable of maintaining a "steady state" with regard to circulatory and respiratory function. According to this standard the pulse frequency should show no tendency to increase above 170 after 6 minutes at a given load, and the respiratory frequency should not exceed 30 in men. Furthermore restitution is considered to be delayed if the pulse frequency does not return to the resting value + 15 within 10 minutes after the work is discontinued. In his material Wahlund finds that the working capacity of healthy men usually amounts to 1200 Kg.M./min. and he considers below 900 to be definitely abnormal.

Since the functional capacity depends upon a number of factors other than the condition of the heart and lungs it is extremely difficult to establish normal values. The total hemoglobin is probably the best correlation factor (Kjellberg, Rudhe, and Sjöstrand, 1949). We have not been able to make this determination, however.

A work capacity of 900 Kg.M./min. has been accepted as a normal value for a man of more than 65 kilograms in this paper since most of our patients have been convalescents. In later tests following recovery 1200 Kg.M./min. has been required. Sjöstrand has required 600 Kg.M./min. as the lower limit

for women of average size.*) So far as we know, no normal standards for tests of this sort have yet been established for children.**) The work test for children on the bicycle ergometer has been intended for the purpose of obtaining a work electrocardiogram, and for the present we have not ventured to assign any decisive significance to their function tests although they were carried out in the same manner as in adults. The pulse and respiratory freqency are significantly higher in children than in adults. A pulse frequency of 180—200 and a respiratory frequency of 40 would seem to be consistent with the "steady state" at least during the first school years. We have repeatedly seen children cycle with a fairly constant respiratory rate of 40, be entirely unaffected, have a normal work electrocardiogram, and return rapidly to the resting values. Children seem as a rule to be capable of 300 Kg.M./min. after 7 years and of 450 Kg.M./min. at 11—12 years.

The Work Electrocardiogram

Using the extremity leads electrocardiograms have been recorded routinely in connection with the function test, both immediately and 4 minutes after the work was discontinued. A recording has also been taken frequently 2 minutes after the work using precordial leads. In a number of other cases the work has been carried out only for the purpose of recording the work electrocardiogram. In these cases a bicycle ergometer has also been used. It can be adjusted for using with children even as young as 5 or 6 years of age by substituting a special children's saddle. The resistance has been varied from 200—300 Kg.M./min. for children to 1500 Kg.M./min. for athletic men. The length of the work period has been 6 minutes, sometimes with several consecutive periods at increasing loads. Small children have been asked to run up and down ordinary stairs. We have made an effort to record the electrocardiogram as soon as possible after the completion of the work, usually 30—45 seconds. This has been possible by attaching the extremity electrodes to the patient during the cycling or before. The electrocardiogram has in these special cases often been taken at shorter intervals: immediately, and at 1, 3, and 5, and sometimes even 10, minutes after the conclusion of the work.

By the use of the resting and work electrocardiograms a conception may be gained of some physiological variations in the electrocardiogram in healthy individuals resulting from various functional states of the heart as a consequence of vegetative and hemodynamic variations. On the other hand, in certain patients with heart disease whose resting electrocardiograms are normal or only questionable it is possible to observe definite pathological changes after

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*) Personal communication.

**) At the close of this work P. O. Åstrand has published a thesis dealing with this point: Experimental studies of the work capacity in relation to sex and age. (Munksgaard, Copenhagen 1952).

work. The bases for interpretation and the application are discussed further in the chapter dealing with the various types of changes.

The Electrocardiogram in Various Modifications of the Horizontal Position

The standard extremity leads have been recorded with the test subject lying on his back, the left and right sides, and on the abdomen. In addition, the patient has often been asked to breathe deeply while lying in the supine position. The position of the trunk has been the main consideration, and the patient has been permitted to place the arms and legs for best relaxation. As a matter of fact the position of the limbs has no effect on the appearance of the curve. The recordings have been relatively free of disturbances. They have been studied in order to evaluate variations, suspected of being the result of changes in the position of the heart.

The Amyl Nitrite Test

In these experiments a resting electrocardiogram has been recorded at first. The patient then inhales deeply two or three times from a crushed ampoule of amyl nitrite. The effect on the electrocardiogram at various frequencies is studied by means of long recordings. These have then been compared with changes in the patient's electrocardiogram which it was thought might be related to sympatheticotonia.

The Ergotamine Test

Ergotamine has been used because of its sympatheticolytic effect, in an attempt to normalize electrocardiograms with changes supposedly due to sympatheticotonia. This point is discussed later. Dihydroergotamine has been given subcutaneously, 0.5 mg. for adults and 0.25 mg. for children. The electrocardiogram has been recorded before, and ½—an hour, 1 hour and often 1½ hours after the injection.

Experimentally Produced Stimulation of the Vagus

In order to study the effect of vagal stimulation on the electrocardiogram, recordings have been made while pressure was applied to the eyeballs or carotid sinus. These patients have also been asked to breathe deeply as a result of which sinus arrhythmia appears. This simple test may often provide certain forms of arrhythmia.

The Clinical Examination

All specialization involves the danger that the individual may be forgotten in the emphasis on one system. This danger is even greater when the interest is absorbed in a special examination. It is still possible to establish the correct

proportions and background of a study only from the patient himself and from the total picture which he presents.

The History

In the taking of the history an attempt has been made to avoid suggestion to the patient. In the case of adults and older children the first question has generally been, "Have you felt the same since you had scarlet fever or not?" If the answer was negative further description has been requested. They have subsequently been asked in particular about the following symptoms — (increased) dyspnea, palpitations, precordial pain or pressure, and marked fatigue. In the case of smaller children it is often possible to obtain a worthwhile history from the mother thanks to her often intuitive understanding of the child. If she reports that the child has been different following scarlet fever, been more tired and fretful than usual, a complication may be suspected. If it subsequently develops that the child seeks quieter forms of play, spontaneously lies down for rest during play, or becomes markedly out of breath there is reason to suspect cardiac complications.

Auscultation

Auscultation of the heart as well as a search for evidence of incompensation should be a fundamental part of the general examination of the patient.

In regard to systolic murmurs it seems to be difficult to differentiate ones due to organic lesions by means of auscultation. The harsh character ascribed to the organic murmurs may even be heard in functional ones both over the pulmonary valve area and the apex. Even if a murmur in a child changes from soft to loud and harsh it is not generally evidence of a pathological change and may be observed even in healthy children according to Epstein. The location also changes in both types, but if the murmur is heard in the left axilla it is regarded as evidence of organic origin according to Wendkos (1947). The variations of the murmurs have been studied in different positions of lying on the back and left side, sitting, standing, and subsequent to work. As a result of an agreement with Ass't. Prof. E. Mannheimer and Dr. T. Möller it has been possible in questionable cases to obtain recording and interpretation of the phonocardiogram at the heart clinic at the Crown Princess Lovisa's Children's Hospital, Stockholm.

Attention has also been given to the strength of the first heart sound. It seems probable that this is partly a result of the closing of the mitral valves at the beginning of ventricular systole (Dock). It would therefore be influenced by the position of the mitral valves just prior to this event and would vary with the time difference between auricular and ventricular systole, i. e. the P—Q time. The first sound will be sharp and clear with a short P—Q time, but moderate prolongation of the P—Q time is associated with a softening of

the first sound. In first and second degree block it changes in strength from beat to beat according to the relationship between P and QRS (Wolfert and Margolies, Levine, Beard and Decherd).

X-ray of the Heart

It has not been possible to make routine x-ray studies of the heart. However, when a murmur has been suspected of being organic and in some of the cases of myocarditis or of other changes in the electrocardiogram the heart volume and configuration has been determined.

CHAPTER 3

General Standards of Interpretation

The Diagnosis and Classification of Myocardial Damage

All electrocardiograms have been interpreted by the author herself on the basis of the literature concerning normal cases as well as myocarditis in various infectious diseases. The greatest emphasis has been placed on serial electrocardiograms and individual interpretation of these. Since the myocarditis of scarlet fever frequently appears later in the illness and is transitory in character in the greatest majority of cases, it is often possible to see changes develop in a normal electrocardiogram and subsequently regress. When alterations in the electrocardiogram appeared during scarlatina, and these were ascribed to myocardial damage, they have been classified as myocarditis. However, it is difficult to establish whether such a change is due to transient toxic damage, bleeding, or structural alterations in the heart. On the other hand if a change in the electrocardiogram remains constant during the entire period of illness and for a long time afterwards, it must be suspected that it is not a result of the scarlet fever but rather suggests heart disease incurred previously or a constitutional anomaly.

The author is aware of our as yet incomplete knowledge. The significance of a number of electrocardiographic alterations is still in question. The nature of the problem has, however, made it necessary to assume a definite position with regard to each case as far as possible. The author has, therefore, attempted to collect the experiences of others as well as her own and has tried new methods of interpretation. There are, of course, still som questionable cases. Amongst those patients with equivocal electrocardiographic changes, there were in some cases symptoms, suggestive of cardiac disease. These cases were allocated to a special group of probable myocarditis. When making a classification all other questionable cases have been assigned to the "non-myocarditis" category. This distinction should therefore not be regarded as conclusive, and it is possible that future experience will require a revision in certain respects. In any case the classification represents a serious effort to achieve a uniform and adequate interpretation.

From the cases which the author has listed as myocarditis, a group with marked electrocardiographic changes has been assembled. More detailed information about this classification is given in a later part of the book. The

criteria employed in evaluting changes in the electrocardiogram vary considerably from author to author, but there is probably little doubt that these more marked changes would be classed even by exacting critics as definitely pathological.

Individual Interpretation of the Electrocardiograms

Serial electrocardiograms can improve the diagnosis of myocarditis by differentiating between current and old myocardial damage. Furthermore, they make it possible to interpret an electrocardiogram both with regard to permissible variations between different individuals and in one and the same individual. Many and large studies of the electrocardiogram in healthy children and adults have given us the limits for normal variation on the basis of statistical treatment. Considerably fewer in number are the studies illucidating the individual's variations from one time of day to another and from day to day (Lewis 1912, Wickström 1932, Grewin 1948, and Simonson et al. 1949). These individual variations seem to be significantly smaller as a rule than those permissible from one person to another. As a result values which are pathological for certain individuals will be found to be within the accepted normal limits. On the other hand, there are quite healthy people, whose variations extend beyond these limits. One of the purposes of this work has been to study these variations in single individuals. This demands, first of all, knowledge as to which physiological factors influence the electrocardiogram and, secondly, experiments to reproduce such variations and to reveal their magnitude in different individuals. The following chapter, number 4, is concerned with the study of such normal variations in the electrocardiogram.

The relationship is analogous with regard to certain disturbances of rhythm, as for example, coronary sinus rhythm. They may sometimes be caused by myocardial involvement, but in other cases they simply signify an increased susceptibility to vegetative influences. Definite lines to follow for interpretation are lacking, and the problem must therefore be considered individually.

Determination of the Range of Individual Variation

It is seemingly impossible to establish generally valid limits with regard to the permissible range of variation in the electrocardiogram of the normal heart, especially during the extraordinary conditions of illness. They vary from individual to individual but of course it should be possible to clarify them experimentally. In the case of questionable pathological changes during an illness, it is helpful to determine whether they can be reproduced later when all other evidence of disease has disappeared and the electrocardiogram has become normal. It has been our principle to use stimuli as physiologically normal as possible in order to produce the functional state which was

suspected of causing the changes. If they have been reproducible it supports the idea that they lie within the individual's normal range of variation and that they may have been due to a functional physiological alteration rather than a myocardial lesion. The procedure is naturally quite different with various types of changes, and a report is given below in connection with the discussion of these changes.

CHAPTER 4

Physiologically Normal Variations in the Electrocardiogram

Introduction

It was formerly believed that a person's electrocardiogram was constant in form. In the supine position and under standard conditions — i. e. fasting and at rest with absence of psychic stimulation — the electrocardiogram of a healthy person actually shows only minor variations, but for practical reasons it is often impossible to obtain such conditions during the recording of the electrocardiogram. The extracardiac factors which influence an electrocardiogram are of two different types: 1) Variations in autonomic tone and hemodynamic changes which directly produce alterations in the electrocardiogram by causing *changes in the functional state of the heart*, and certain rhythm disturbances, 2) Changes in the degree of inflation of the lungs and abdominal viscera which by *changing the position of the heart* indirectly produce alterations in the electrocardiogram by changing the position of the electrodes in relation to the heart.

I. Variations due to Changes in Autonomic Tone

The variations in the electrocardiogram which are associated with changes in autonomic tone are of two different types. These are labelled "sympathicotonic" or "vagotonic" according to which of the two systems is in predominance. The relationship between vegetative tone and changes in the electrocardiogram is probably complicated. These alterations must be ascribed, not only to accelerator or vagal effects on the heart, but also to the action of adrenaline and acetylcholine. In addition, changes due simply to variations in frequency must be reckoned with, quite apart from the autonomic influences. For practical reasons, however, it would seem to be justifiable to make the distinction in order to differentiate between the two types.

The magnitude of these changes varies from one healthy individual to another. The considerable variations in conduction time seen in certain — although not all — highly trained athletes and the different extents to which S—T and T vary with changes in frequency are illustrative. In addition, a

person with an acute infectious disease, even if the heart is not involved, would be expected to show more marked changes in the electrocardiogram than normally as a result of the considerable variations between the sympatheticotonia of the febrile period and the vagotonia of convalescence.

A. Electrocardiographic Changes of the Sympatheticotonic Type

The sympatheticotonia seen with fever in the initial stage of the infection or present even later in anxious or vegetatively over-sensitive patients, is associated with lowering of the S—T segment with or without flattening of the T wave as occurs in myocardial damage. This is of the highest importance from the standpoint of differential diagnosis. Other findings are an increase in frequency, high pointed auricular waves, and a relatively short conduction time.

The degree to which the length of the cardiac cycle affects the appearence of the S—T segment and the T waves is sometimes well illustrated by their deformation in auricular, premature systoles (figure 12) and by their appearance in fibrillation. In high-frequency fibrillations deformation of the terminal complex, when it occurs, is most pronounced in connection with the shorter R—R intervals, and less pronunced with the longer in which the terminal complex may be entirely normal. On the other hand, in very slow fibrillations the changes are greatest with the longer — abnormally long — systolic intervals. In the first case the length of the recovery time would seem to be the deciding factor; and in the latter, whether anoxemia has time to develop or not. Of course such observations in cases of heart disease cannot be applied directly to normal subjects, but it is, however, justifiable to use them as an illustration of the effect of the frequency, per se, on the terminal deflection.

Vegetative tone changes are usually reflected in the frequency although the latter may also be affected by hemodynamic factors. As Sjöstrand (1950) has shown experimentally on 70 normal test subjects, using scopolamine, amyl nitrite, as well as physical exertion, the T waves seem to diminish in amplitude in a linear relation to the increase in frequency, and they seem to follow the same regression line independent of the mechanism. In the presence of marked amyl nitrite effect, as well as when the body position of the patient is changed so that definite displacement of blood takes place within the body, the flattening of the T waves is greater, however, than that expected from the coincident increase in frequency. Immediately after work an increase in amplitude occurs in spite of the increase in frequency, which may also be ascribed to certain hemodynamic factors.

Corresponding considerations apply to the S—T changes, although these follow a curved line. This displays an elevation at first and subsequently a more and more pronounced depression with rising pulse frequency, according to Sjöstrand (1950). He also found, that the lowering of the S—T segment

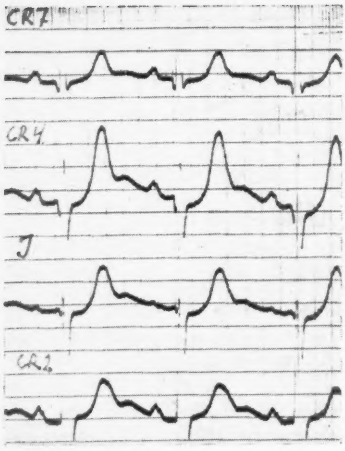


Fig. 1. *S-T depression and marked positive after-potential.*

(Precordial leads CR_7, CR_1, J, and CR_2 recorded 3 minutes after work.)

The T-P and the P-Q intervals are each raised by the positive after-potential and the S-T segment is lowered in relation to the P-Q level.

might be a relative depression due to an elevation of the base line (P—Q interval) by a positive after-potential. This S—T change is usually of a definite type with a "low junction". Figure 1 illustrates the chest lead picture taken at follow-up examination 3 minutes after work in a boy with a marked positive after-potential. This seems to elevate the T—P as well as the P—Q intervals, the S—T segment consequently appearing lowered.

For practical purposes the frequency relationship should usually be a good basis when resting electrocardiograms taken with the patient in recumbancy are to be compared and interpreted. Changes in autonomic tone should be considered, however, even when the frequency is unaltered. Such changes may be reflected in the T wave and S—T segment as illustrated in the following case: This was a 4 year old boy (record no. 2584/49 fig. 2) who showed marked positive after-potential and depression of S—T with a high, positive P_2 in one electrocardiogram, in the next one a low broad T_2, while in the third one a definitely positive T_2 was accompanied by a weekly positive P_2. The frequency was 120 at all three times. The P waves, however, were suggestive of a sympatheticotonia in the two first electrocardiograms and it seems probable that this also caused the flattening of T_2 and depression of S—T.

For purposes of differential diagnosis we have proceeded according to the description below.

a.) *Changes of short duration*

The amyl nitrite test.

In changes of short duration of sympatheticotonic type we checked the temperature chart to see if there was any fever at the time of the recording. If such was the case there is definite suspicion that merely a sympatheticotonia was the cause. On the other hand a fever may accompany a myocarditis.

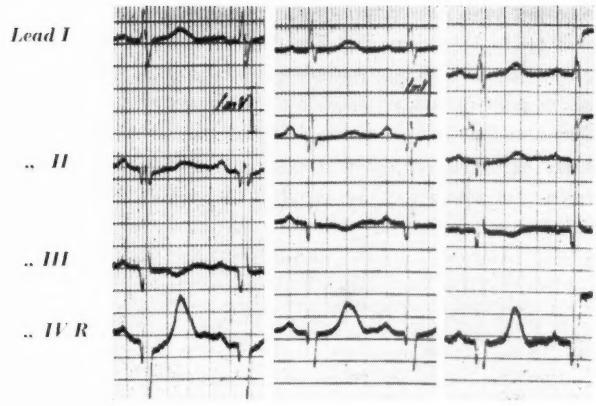


Fig. 2. *S-T and T changes with the same heart rate, possibly due to autonomic influences.* (Record no. 2584/49.)

a. High, positive P_2 and P_3. Marked positive after-potential and depression of the S-T segment in leads II, III, and IVR. T_2 is lowered.

b. High, positive P_2 and P_3. The S-T segments are isoelectric and the after-potential is not marked. T_2 is lowered.

c. Weakly positive P_2, and diphasic P_3. T_2 is definitely positive.

The fever sympatheticotonia seen in our milder cases of scarlet fever usually subsided rapidly and coincidentally their electrocardiographic changes. Such changes in the electrocardiogram may also be transitory if they were the result of a temporary anxiety. It must be remembered, however, that a myocarditis in itself may cause a tachycardia. Latent myocardial damage, not apparent in the electrocardiogram at lower frequencies, may also become manifest as a result of the increased stress of tachycardia.

In such cases as mentioned above we have at suggestion of T. Sjöstrand made an amyl nitrite test on the patient when the electrocardiogram subsequently became normal, either during convalescence or, preferably, at the time of a later check-up. This use of amyl nitrite for making a differential diagnosis is namely based on the assumption that a myocarditis would, in all probability, have healed. The inhalation of amyl nitrite would seem to set in motion a complex mechanism. Vasodilatation, a drop in aortic pressure and raised accelerator tone are typical features. (See inter alia Nordenfelt and his bibliography.) To avoid major shifts in the distribution of the blood with greatly lowered S—T and T, an effort was made to ajust the amount inhaled

to a level, corresponding with only a moderate rise in pulse rate. As the frequencies vary, different types of S—T and T changes appear. The alterations seen at any given frequency during the rising phase do not generally correspond in type or degree to those observed at the same frequency during the falling phase. All these findings, however, must be considered as normal in any particular, healthy patient.

The physiological variations in S—T and T seem to be closely related to the heart frequency as discussed above. If the changes are now reproduced at the same frequency at which they appeared in the suspicious electrocardiogram it is regarded as an indication that they may have been the result of a sympatheticotonia, although the clinical picture must also be taken into consideration before making the ultimate decision. If the changes do not reappear, if they are of a different type than previously, or if they appear only at a significantly higher frequency than in the earlier electrocardiogram, it is then probable that they were the result of a transitory myocardial lesion. By means of the same experiment, sympatheticotonic shortening of the P—Q time and elevation of the P wave is illustrated. The test seems to be of special value since it illustrates the way in which S—T and T display varying sensitivity to an increase in frequency in various individuals.

b.) *Persistent or recurrent changes.*

1. The ergotamine test.

In cases of prolonged or recurrent changes in the S—T segment and/or the T waves which might be related to a constitutional sympatheticotonia there is another diagnostic procedure at our disposal, the ergotamine test. According to Nordenfelt and several later authors a normalization of the electrocardiogram in response to ergotamine suggests that the changes were not due to organic damage but rather to a sympatheticotonia. To judge, however, from the studies of Scherf and Schlachmann (1949) this statement would seem to be subject to modification. They have discovered that even definite organic changes in the T wave may be normalized by ergotamine. According to Kühns it would appear that both the coronary circulation and metabolism are altered by ergotamine, and he feels that he has shown both in animal experiments and clinically that changes in the electrocardiogram due to myocardial or coronary disease may be normalized by ergotamine. Since ergotamine has not only a sympatheticolytic effect but also a constricting one on smooth muscle, its action on the heart is not completely known and difficult to evaluate. It seems reasonable to suppose that pathologically flat T waves as well as pathologically prolonged conduction times could be functionally variable. The normalization may even be only apparent and the explanation be that the recording is made from large surfaces of the heart and is a summation of potential variations within the surfaces. Positive potentials in the vicinity can

compensate for a negative T wave within one portion of such a recording surface. An increase in the positive potential in the area might even be thought to compensate for a negative potential which had previously appeared in the recording. It would thus appear that a negative potential had diminished although it is in reality unchanged. Ergotamine has been used in some cases as a supplementary test since it, however, gives an idea of the variability of the electrocardiogram. Our experiences with the ergotamine test are discussed further in the chapter on S—T and T changes.

2. Prolonged Observation

Another helpful course in making a differential diagnosis between the electrocardiographic change due to a sympatheticotonia and one due to a myocarditis is simply prolonged observation of the patient, preferably for at least a year. The psychic factor of tension or anxiety which inclines toward sympatheticotonia not uncommonly disappears after repeated tests as the patient becomes accustomed to the apparatus and the technician. An amyl nitrite test after the electrocardiogram has normalized may verify the lability. If the changes are then found to be consistently dependent on the frequency with normalization at lower frequencies, it is probable that they are related to autonomic variations. These vegetatively labile subjects are found to be in good condition both according to their history and the functional test, and their work electrocardiograms show either changes entirely due to the frequency or none at all. By means of such continued observation of the patient the diagnosis can usually be confirmed although it is unfortunately delayed.

B. Electrocardiographic Changes of the Vagotonic Type

A vagotonia, whether of constitutional or post-infectious origin, consistently causes a low frequency, low P waves, a relatively long, and sometimes abnormally long, P—Q time, an isoelectric or slightly elevated S—T segment, as well as highly positive T waves. It is not uncommon to see disturbances of rhythm in such individuals — sometimes a wandering pacemaker, sometimes escaped beats. The prolonged conduction time and the ectopic rhythm may cause difficulties in the differential diagnosis between vagotonia and myocarditis. Studies of the electrocardiogram after work and during experimentally produced vagotonia can help in making the decision.

a.) *Prolonged A—V Conduction Times*

1. The Work Electrocardiogram

When there is doubt as to whether a temporary A—V prolongation in a symptom-free patient is due to a vagotonia or a myocarditis the work electrocardiogram is often of value. This also holds in cases with permanent prolongation (Reindell et al.). Such a "stress test" should of course not be per-

formed until the changes have regressed or at least been present for some time.

A series of rest and work electrocardiograms may *reveal evidence of a myocarditis;* firstly, if the suspicious P—Q value is not reproducible and the variations are not as great as those observed earlier; and, secondly, if the P—Q time is prolonged with a rise in pulse rate immediately after work. The latter finding is generally regarded as pathological and indicative of abnormal fatigability. The normal reaction in this phase is a shortening of 0.01—0.03 seconds. It should of course be observed in this connection that it is not the changes in the P wave which produce the apparent changes in the conduction time. On the other hand, pathologically prolonged conduction times are not uncommonly shortened during exertion. Neither this nor marked variability rule out an acute myocarditis, however, but it may be regarded as a functionally favorable sign in general. The range of variation at different frequencies should not ordinarily exceed 0.05 seconds. Greater variations are suspicious but are sometimes seen in the absence of heart disease in well trained athletes and people unusually subject to vagotonia, as for example patients with duodenal ulcer (Biörck et al. 1948).

A work electrocardiogram may also *make a myocarditis less probable.* This cannot be done, however, until a sufficiently long time has elapsed so that a possible myocarditis would, in all probability, have regressed — in other words after one or two years. In this investigation most of the A-V disturbances as a result of unequivocal myocarditis have been normalized within this period. If the suspicious value can then be reproduced it probably belongs to the individual's normal variation with the reservation that persistent myocardial damage may have developed during the illness. The clinical picture is thus naturally of the highest importance for forming an opinion. For actual procedure see further the chapter on disturbances of A—V conduction.

2. Electrocardiogram during Deep Breathing

The sinus arrhythmia with deep breathing may be accompanied by large changes in the conduction time (Ljung 1951). This may help to illustrate the considerable variations met with in some apparently normal individuals.

b.) *Disturbances of Rhythm*

1. The Work Electrocardiogram

A slowing of frequency resulting from vagal stimulation has a greater effect on the sino-auricular node than on the lower ectopic centers in the auricle and the A-V node (Meek and Eyster, von Hoesslin, Ritchie). If the frequency in these lower centers then exceeds that in the S-A node they assume the pacemaker function. But sinus arrest and abnormal foci of stimulation can also result in ectopic rhythms. In many of these cases the etiology is therefore doubt-

ful. It is of interest to see the effect of work on an ectopic rhythm. In ectopic rhythms due simply to neurogenic factors the sinus rhythm will be reestablished in at least some phase of the work electrocardiogram. Of course it is possible that the same thing may occur in the presence of incomplete sinus arrest or abnormal foci of stimulation with lower frequencies, but this is less likely. In any case the work electrocardiogram demonstrates whether the S-A node is still capable of assuming the pacemaker function or not. Subjects with a constitutional tendency to nodal rhythm who are found to have a sinus rhythm during rest often display the nodal rhythm again during the vagotonic phase of the work electrocardiogram. The relationship of the escaped beats to work and rest is also of value in interpreting their nature.

2. The Electrocardiogram during Experimental Stimulation of the Vagus and Deep Breathing

In patients who have had disturbances of rhythm of purely vagotonic origin it should be possible to reproduce these by means of various factors which cause an increase in vagus tone and decreased heart frequency. By means of simple measures such as deep breathing, pressure on the eyeballs, or pressure on the carotid sinus it has often been possible to reproduce these wanderings of the pacemaker and escaped beats at follow-up examinations.

II. Changes in the Electrocardiogram Resulting from Changes of Position

A. *Spontaneous Positional Changes in the Electrocardiogram*

Changes of position of the heart and its electrical axis may occur as a result of changes in the degree of inflation of the abdominal viscera and lungs. They manifest themselves in the electrocardiogram as changes in the ventricular complex and its electrical axis and amplitude. The T waves seem to change simultaneously although often to a lesser extent than QRS. The variations are probably explicable on the basis of changes in the relationships of the electrodes to the heart as a result of which the components from the right and left ventricles are projected in the electrocardiogram in changed relationship to each other. As a rule, the electrical axis of the T wave and that of the ventricular complex are displaced in the same direction. T_1 is thus usually lowered or flattened coincident with deviation of the electrical axis of the ventricular complex to the right. This is naturally most obvious in children, where the amplitude of the T wave is much lower in the right than in the left ventricular curve. When the changes in QRS and T take place in opposite directions this may be the result from additional effect of altered vegetative tone or myocardial damage.

B. Experimentally Produced Positional Changes in the Electrocardiogram

It has been known for a long time that changes in the body position are accompanied by changes in the electrocardiogram (Einthoven et al.). Heretofore the chief interest has been directed towards the changes consequent to the lying, sitting and standing positions. These are probably largely due to changed hemodynamic and vegetative relationships and to a lesser degree to alterations in the position of the heart. The changes accompanying the various phases of respiration result both from changes in the position of the heart as well as autonomic influences.

In addition to the actual displacement of the heart, alterations of conductivity in its surroundings may have an effect. According to Burger (1926) and Klink (1933), however, the position of the ventricles of the heart in relationship to the frontal plane has a determining effect on the appearance of the electrocardiogram. Lepeschkin treats these "positional changes" in detail in his text book to which the reader is refered for further literature on this point. In general the right side lying position as well as the prone position cause a displacement to the left of the electrical axis and the left side lying position a displacement to the right. Changes in amplitude may also occur.

C. Earlier Employment of Changes in Position for Diagnosis

The variations in the electrocardiogram concomitant with various modifications of the horizontal position (page 20) have been used earlier in the diagnosis of adhesive pericarditis. The diagnosis is felt to be strengthened if the expected variations fail to occur. Littman has also used the prone position in the evaluation of extreme normal variations of the T wave. Beyond this it does not appear that these alterations have been used for diagnostic purposes.

Respiratory changes in T_3 have been used for the interpretation of a negative T_3. According to Parade (1939) a negative T_3 which is due to horizontal position of the heart will disappear with deep inspiration, but if it is due to heart disease it will remain negative. According to Hemmersbach 1941 (cit. Lepeschkin), however, this is not always true. With deep breathing plus the Valsalva maneuver Klink has also observed a negative T_3 become positive in cases of cardiac hypertrophy as well as in those of simple horizontal position of the heart. It appears, however, that no corresponding efforts have been made to interpret consecutive changes of T_3 in serial electrocardiograms.

D. Own Employment of Positional Changes for Diagnosis

The combination of right axis deviation and low or isoelectric T_1 in children in itself signifies no abnormality. It is only changes in the electrical axis or lowering of T or both, occuring from one electrocardiogram to another during

a series of electrocardiograms, which are suspicious and may be evidence of myocardial damage. So far as T_3 is concerned it spontaneously shows such large variations anyway that it seldom is assigned any diagnostic significance. However, there are probably types of myocardial damage which are best, if not only, seen in lead III (Buchem and Daniels). Those changes, seen with variations in position of the heart and possessing definite characteristics, must be carefully distinguished from those arising from myocardial disease.

Of course, in the same individual, quite separate factors such as myocardial disease and change of position may operate consecutively, when they are obviously mutually independent, or simultaneously, when they may or may not be independent of each other. In the latter case, the myocardial disease may, due for example to dilatation, have led to positional changes.

In this study electrocardiograms have been recorded in the various horizontal positions to differentiate changes possibly due to position from those due to myocardial disease. Thus the variations in any individual due to different positions of the heart may be appreciated. If the change does not reappear it would seem that the cause was a myocardial damage. On the other hand, if reproducible, earlier observed combination of changes may have been due to positional alterations and does not justify the diagnosis of myocarditis. This, of course, is on the assumption that the examination has been performed after recovery. Otherwise one must count upon that a myocarditis might be evident only in a certain position due to the relationship of the electrodes to the heart lesion. In addition, a permanent change may because of its circumscribed nature show up only with the heart in certain positions.

It seems hardly probable that it would be possible to reproduce the changes which resulted from myocarditis by means of alterations in the position of the heart after recovery. This is especially true since changes of position and myocarditis affect the QRS complex and the T waves in different ways and to different degrees, and seems possible in but one type of cases: where myocarditis only affects the electrocardiogram in that it involves an altered anatomical relationship to the electrodes, due for example to dilatation. A myocarditis of this kind would hardly seem recognisable with the methods of investigation here used.

Myocardial damage, which affects the T waves, may coincide with changes in QRS and T due to altered position. In this case, certain QRS and T alterations should of course be reproducible at the follow-up examination. However, the earlier seen combination of QRS and T changes does not reappear.

For further information regarding the employment of the method see chapter 10: II Variations accompanying changes of position.

CHAPTER 5

Disturbances of Sinus Rhythm

Frequency

In electrocardiograms which appeared to be normal in other respects no studies of the frequency have been made. In the case of subjects with wide variability of the pulse, as for example children, it would seem to be best to take the pulse while the patient is asleep if any conclusion is to be drawn on the basis of the frequency. Sinus tachycardia and sinus bradycardia will be mentioned only when observed in association with other electrocardiographic changes.

As expected, sinus arrhythmia has been common in our material consisting as it does mostly of young subjects and convalescents. Only those considered to be due to sinus arrest rather than respiration have been especially studied.

Sinus arrest

By the term sinus arrest (de Graff et al.) is meant that condition in which the impulses from the sino-auricular node partially or wholly fail to stimulate the auricles. This condition, usually under the name of sino-auricular block, has been described by several authors (Wenckebach, Spühler, Froment et al., Andersen, Jervell, Winton, Tournaire, Sabattie et al., and Salvesen). In addition to interference with the conduction of the impulse from the S-A node to the auricles it would seem possible that sinus arrest could occur as a result of a disturbance in the development of the impulse in the S-A node itself (Jervell, Froment et al.).Since the impulse formation can not be recorded in the electrocardiogram this aberration can only be diagnosed by indirect methods, on the basis of the appearance of pauses in the sinus rhythm or ectopic rhythms of certain characters. Thus since the disturbance can frequently not be localized the term sinus arrest would seem to be most suitable. Disturbances in the conduction pathway between the sino-auricular node and the auricles may of course manifest themselves in changes in the auricular waves as the impulse passes through the auricles by other routes (Rothberger and Scherf). In this chapter, however, only sinus arrest will be discussed, while changes in the auricular complex will be discussed in a later chapter (see "Changes in the P waves").

Sinus arrest of varying degree is described — intermittent and constant, incomplete and complete. There are two kinds of incomplete sino-auricular block (Wenckebach), corresponding to the grade II atrioventricular blocks of the Wenckebach and Mobitz types. In the first type the resistance gradually increases with the P-P interval until it is just doubled in length thus corresponding to a dropped impulse. The series it then repeated. In the latter type regular P-P intervals are occasionally interrupted by a doubled interval.

As the P-P intervals vary the similarity to respiratory sinus arrhythmia may be confusing. Such cases are easily overlooked (Sabattie) but may be revealed by simultaneously recording of the respiration. The work electrocardiogram may also be of value since the respiratory arrhythmia decreases or disappears during and immediately after work. In the relatively short pauses seen with incomplete block an ectopic pacemaker rarely comes in, but escaped beats may be seen. In complete blocking it seems that an ectopic pacemaker usually appears quickly. Nodal as well as ventricular rhythms develop in such cases. A constant nodal rhythm in which the sinus rhythm can not be experimentally brought out would seem to be evidence of complete sinus arrest.

Etiology and Occurrence

According to Sabattie sino-auricular block may be due to vagotonia, arteriosclerosis, or digitalis. Spühler, who has reported cases of sino-auricular, intraventricular, as well as sino-ventricular conduction disturbances, believes that myocarditis can also cause these states and that they are pathological. He also claims that the blood supply to the sino-auricular node is a very important factor. Winton, on the contrary, who has described this disturbance in organic heart disease, attributes it to vagotonia and does not feel that it has an independent pathological significance. According to Nadrai sino-auricular block is not uncommon with infections in children and a case has been described by Blumberger, and a doubtful one by Wickström, in scarlet fever.

In this study sinus arrest has been observed only once. This was the case of a 3 year old boy (Record no. 1659/49) with a very mild case of scarlet fever. The routine electrocardiogram in the third week showed a markedly irregular sinus rhythm with a doubling of the intervals. The control electrocardiogram taken in rest showed a nodal rhythm alternating with a sinus rhythm (figure 3 a). The sinus rhythm was highly irregular but synchronous with the respiration which was noted by the calibration marker on the electrocardiograph. The shortest P-P intervals ocurred with inspiration. P_1 and P_2 were positive while P_3 tended to be negative. The P-Q interval was 0.13—0.15 seconds and the average frequency was 55 per minute. Alternating with this rhythm was an ectopic rhythm, of the upper A-V nodal or coronary sinus type, with negative P waves in all 3 leads and a P-Q interval equal to 0.11—0.12 seconds. This was called ectopic rhythm type 1 in this patient. The rhythm was somewhat irregular although less so than the sinus rhythm, and the frequency

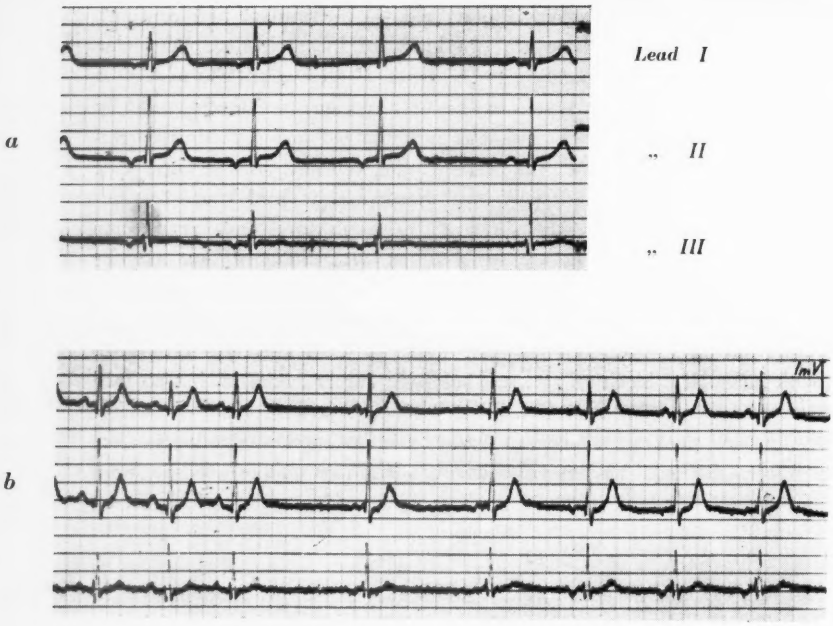


Fig. 3. *Sinus arrest and 2 ectopic rhythms.* (Record no. 1659/49, leads I—III.)

a. During rest, showing transition between ectopic rhythm type I of activ nature with a frequency of about 100 and sinus rhythm with a frequency of about 55.

b. Immediately after work the high-frequency sinus rhythm ceases. After a doubled systolic interval ectopic rhythm type 2 (see text) appears.

c. A few seconds after curve b. a high-frequency sinus rhythm reappears.

d. The curve is recorded 3 to 4 minutes after work and shows ectopic rhythm type 1 with marked respiratory arrhythmia. Notice the constant P waves! (Each inspiration is marked by the calibration test.)

was about 100. This ectopic rhythm would therefore seem to be of the active type and indicative of irration.

Immediately after work (figure 3 b) 14 beats of entirely regular sinus rhythm were recorded. The frequency was 120 with highly positive P waves and P-Q equal to 0.12—0.13 seconds. Subsequently the P-P interval doubled and after that 14 contractions were recorded with a positive P_1, a diphasic or negative P_2 and a negative P_3 with a P-Q interval of approximately 0.10 seconds. The frequency was low at first but increased, and after the third beat it was regular at about 105. This particular ectopic rhythm is labelled type 2. It seems to start from the coronary sinus area or probably the periphery of the S-A node. After an unchanged P-P interval sinus rhythm reappeared with a frequency diminishing from 135—120 per minute (figure 3 c).

The suddenly occurring pause (figure 3 b) in a regular high frequency sinus rhythm is strongly suggestive of sinus arrest. The interfering ectopic rhythm must thus have been passive in nature. This agrees with the fact that both the preceeding and following sinus rhythms were of higher frequency. In a subsequent electrocardiogram with recording of the respiration, ectopic rhythm type 2 appeared with a frequency of about 70 and with small respiratory variations. Ectopic rhythm type 1 reappeared 3—4 minutes after work (figure 3 d) with a marked respiratory arrhythmia and an average frequency of 80 per minute. It is readily seen in this curve that the auricular waves are not affected by respiration or changes in frequency. For this reason it would seem that the changes in the P waves are actually a result of a shifting of the pacemaker from one focus to another. If the sinus impulse should follow a varying course through the auricular wall it might offer another possible explanation of the change in the P waves. However, evidence of a changing pacemaker is also found in the fact that sinus rhythm and ectopic rhythm type 1 occur in the resting electrocardiograms at obviously different frequencies and in the fact that there is a varying respiratory sensitivity with arrhythmia of varying degree in the 3 different foci.

It would thus seem that an incomplete, intermittent sinus arrest is present as well as two different auricular pacemakers. This sinus arrest occurring with tachycardia immediately after work may possibly be ascribed to sudden vagus stimulation, but it was more probably due to organic heart disease, a myocarditis. The presence of two ectopic rhythms, partly of the active type, also speaks in favour of a pathological state.

This boy has been free of symptoms. At a follow-up examination one year later he showed ectopic rhythm type 1 with marked respiratory changes during rest. It was only after work that a markedly irregular sinus rhythm appeared

which later alternated with ectopic rhythms of both types 1 and 2. A sinus arrest with doubled intervals did not appear, however. This phenomenon with two different types of ectopic rhythm still seems suspicious but would not seem to justify definite conclusions. On the other hand, the patient's earlier sinus arrest and active ectopic rhythm has been interpreted as indicative of a myocarditis.

CHAPTER 6

Coronary Sinus Rhythms and Auriculoventricular Nodal Rhythms

Definition

In this chapter the classical form of nodal rhythm originating from the A-V node will be considered as well as the coronary sinus rhythm (Zahn 1913, Wenckebach and Winterberg 1927, Scherf 1944, and Scherf and Harris 1946), since these are related both as to origin, mechanism and appearance of inverted P waves. The coronary sinus area is even regarded as an offshoot of the A-V node by some authors. On the other hand, Rothberger (1931) feels that the so-called coronary sinus rhythms probably come from offshoots of the sino-auricular node.

These apparently diverging conceptions would seem only to present different points of view. Embryologically the coronary sinus region, just as the auricular part of the A-V node, is separated from the S-A node as a result of the development of the auricles. Anatomical and histological studies in man (Kung 1930) have shown that so-called "bridging fibres" run from the coronary sinus region as well as from the right and left sides of the auricular septum to the auricular part of the A-V node. These fibres differ in their structure from the other types of tissue in the A-V node itself and are more similar to those in the S-A node and the auricular musculature in general. The coronary sinus region is notable for its wealth of ganglion cells.

Embryologically and histologically speaking, the coronary sinus region approximates to the S-A node. Its high degree of automaticity is therefore to be expected and the large number of ganglion cells explains its sensitiveness to autonomic impulses. Anatomically speaking, however, the coronary sinus area lies in close association with the A-V node. This explains why, with upper nodal and coronary sinus rhythms, there is the same time relationship between the auricular and ventricular systoles and the same type of P wave — the latter determined by the course of the impulse through the auricles.

In this connection it is worth pointing out that the auricular part of the A-V node is embryologically, structurally and functionally quite different from the ventricular part. This is seldom considered clinically.

A genuine atrioventricular nodal rhythm, as we know, is recognized by the negative P_2, and possibly negative P_1, plus a shortened, and possibly negative, P-R interval. The presence of the negative interval depends upon how far distal the impulse origin is located. In the more proximally located coronary sinus area the P waves are also negative while the P-Q time is only slightly shortened if at all (Zahn 1913). Therefore, the borderline between a coronary sinus and an upper nodal rhythm must be ill-defined.

The relationship of the P-Q intervals to each other, however, is only indicative of the site of the pacemaker on the condition that conduction is intact (Langendorff et al. 1944, Scherf and Harris 1946). Just as the P-Q time decreases as the pacemaker is located more distally the sensitivity to nervous stimuli is also decreased. It is thus greatest in the coronary sinus area. As a general rule the frequency of the impulse also diminshes the further the distance from the sino-auricular node.

A negative P wave may also be present with an intraauricular conduction disturbance in the presence of a continued sinus rhythm (Rothberger and Scherf). This possibility has been considered but does not seem to have been present in any of these cases. Simultaneously with a change in the P wave the rate and rhythm have also changed, as is characteristically the case with a migratory pacemaker. With only one exception (case 3), the P waves have also had the appearance described with coronary sinus or A-V nodal rhythms.

Pathogenesis

The auriculoventricular node and its offshoots normally possess the capacity for stimulation and may be regarded as effective substitutes for the sino-auricular node. However, pathological processes in the A-V area can also produce ectopic rhythms due to irritation. The A-V nodal and coronary sinus rhythms — here shortly called nodal rhythms — are, like other ectopic rhythms, therefore usually classified as active or passive with reference to their pathological significance.

In the *active* type the nodal rhythm appears as a result of an irritation. This causes an increased frequency in the giving of the impulse, and as a result the ectopic pacemaker displaces the S-A node. An active nodal rhythm is to be regarded as pathological and should be suspected in cases of nodal rhythms of high frequency.

On the other hand, the *passive* forms of nodal rhythms appear during blocking of the impulse from the S-A node or inhibition of its activity with the result that the frequency is lowered to less than that of the pacemaker in the A-V node. The nodal rhythm thus develops as a *substitute* and in itself is physiologically normal. This may be regarded as a form of defense in contrast to the active type, which is a form of attack. The borderline between

passive nodal rhythm and escaped beats is ill-defined. When occasionally several escaped beats, separated by regular intervals, follow each other in succession. this may also be grouped among the ectopic rhythms.

On the other hand, the process causing the blocking of the sinus may be pathological in certain cases. As a rule, however, inhibition of the S-A node is vagal in origin. Since the effect of the vagus diminishes distally it is considerably less in the coronary sinus area and at the A-V node than at the S-A node. An ectopic pacemaker may thus temporarily develop a higher frequency than the S-A node and take over the direction of the heart. In animals Ganter and Zahn 1912 showed, that cooling of the S-A node shifted the pacemaker from the S-A node to the A-V node. The same effect was produced by vagus stimulation, acccording to Meek and Eyster 1914. In some cases only a small wandering occurred within the S-A node, often as transitional forms. There is probably no essential difference between these ectopic pacemakers. Ritchie and van Hoesslin have had similar experiences in normal human subjects, as has Wilson in pathological conditions. Lewis 1920 showed, that shifts occurred following changes in frequency. These shifts take place even more readily if there is only a small difference in the frequencies from the beginning (Rothberger). A frequently recurrent alternation, an interference, may thus take place between sinus rhythm and nodal rhythm and is probably a normal phenomenon in certain subjects.

Partial Interference

When impulses arise simultaneously from the S-A node and the A-V node the auricle may be activated from the S-A node while the ventricle is stimulated by the A-V node. (This would seem to be possible only if the center is in a lower portion of the A-V node. From the upper region or the coronary sinus area the impulse reaches the auricles considerably before the ventricles. and it either controls the heart fully or has no effect.) In this way electrocardiograms may be seen with positive P waves at a varying distance from the QRS complex — shortly before, or partially blending with the ventricular complex, the relationship varying from one systole to the next. A wandering pacemaker may thus be simulated as Wenckebach and Winterberg have pointed out. In such cases there is partial interference between the sinus and the nodal rhythm, limited to the ventricles, while the auricles are always controlled by the S-A node. I should like to call this phenomenon, which also has its equivalent in other types of rhythm disturbances, partial interference. (Figure 8) The dissociation between the auricles and the ventricles which appears here in certain beats is purely a coincidence and secondary to the interference. This phenomenon must be clearly distinguished from dissociation with interference. which will be described below.

Interference Dissociation

Interference dissociation (Mobitz 1923), or dissociation with interference, is regarded as occurring, when an ectopic pacemaker has developed a higher frequency than the S-A node and assumes control but as a result of retrograde blocking does not reach the auricle. The latter is therefore always activated by the S-A node, and a dissociation between the auricles and the ventricles takes place. On the other hand, the anterograde conduction is intact, and when the sinus impulse reaches nonrefractory ventricular tissue it is conducted and interference occurs. The dissociation as a result of retrograde blocking is the primary feature here. The interference is secondary and simply due to the time relationship between the two rhythms in certain instances.

Earlier Experiences and Interpretation

It is to be expected that nodal rhythms will be found both in healthy subjects (Hafkesbring, Graybiel et al.) and patients with heart disease (see amongst others, Scherf and Harris, Flaxman, Langendorff et al. Danielpolu, Wilson). The interpretation in cases with potential heart disease such as rheumatic fever, scarlet fever, and other infectious diseases, is important and has varied in the literature. Nodal rhythms seem to have been assigned a pathological significance as a rule (Filberbaum, Cohn, Spang and Welsh, Steinmann). Even if they have not been diagnosed as myocarditis they have been considered as alterations in connection with the illness (Roelsen, Pardee, Sokolow). Klemola and Wickström consider only those nodal rhythms with evidence of active heterotopia as pathological. Klemola examines with the aid of the work electrocardiogram. Kienle has also noted the importance of this method in the interpretation of both wandering pacemakers and A-V rhythms. If the change remains or increases after exertion he regards it as evidence of damage — hypoxemic or toxic-infectious.

The Author's Basis of Interpretation

If one wishes to take a position with regard to the significance of a coronary sinus or A-V nodal rhythm it is necessary to analyse each case as to whether it is active or passive in character and as to its time relationship with scarlet fever.

The most significant points in this consideration are the frequency of the ectopic pacemaker in relation to that of the S-A node and the transition between the two rhythms. The time relationship between the impulse formation in the S-A node and that in the ectopic center forms an important part of the discussion, as do differences in conduction time from the pacemakers to the auricle. This must complicate the transition from one pacemaker to another

so that the one which momentarily has the higher frequency does not always immediately assume control since its impulses may fall in the refractory periods. On the other hand, a temporary interference between the rhythms may arise if the impulse from the one of lower frequency happens to arrive in the non-refractory period. It is not likely, however, that any great time difference would exist between different physiologically normal pacemakers since the same hemodynamic and neurogenic mechanisms should regulate all of them.

The follow-up examinations have assumed great significance since at this time an effort has been made to produce and study these transitions experimentally. Alterations in frequency and vegetative tone have been produced by means of pressure on the eye balls or the external carotids as well as by deep breathing. Work electrocardiograms have been taken and recordings in the sitting and standing position in some cases.

The work electrocardiogram must be interpreted with reference to the changing vegetative phases following work. A constant A-V rhythm would seem to indicate a pathological process in any case — a lesion in the S-A node with a passive, nodal rhythm or possibly constant irritation with an active one. No cases of this type, however, have been observed in this study, and the sinus rhythm has always appeared in some phase of the work electrocardiogram. A nodal rhythm appearing during work or in the earliest or sympatheticotonic phase immediately following work is probably an indication of a pathological process — a focus of irritation or damage to the S-A node.

A nodal rhythm caused by a myocarditis will probably have vanished by the time of a later examination. On the other hand it should be possible to reproduce a physiologically normal passive nodal rhythm on a later occasion if it was not the result of extreme vegetative lability during the illness. This of course is based on the assumption that the sinus frequency is depressed below that of the A-V node at the examination. A minor stimulation of the vagus might possibly result in limited shifting to center in the periphery of the sino-auricular node — a perisinus rhythm (chapter 7). These perisinus rhythms may also be seen during the transition from a sinus rhythm to a coronary sinus rhythm.

The simultaneous occurrence of premature systoles and an ectopic rhythm from the same focus seems to be especially interesting. Although the appearance of premature beats with fixed coupling is not regarded as being definite sign of heart disease, it is, however, an indication of active heterotopia, a functionally valueless stimulation which distinguishes it from the physiologically normal passive nodal rhythms. If an insulative blocking of the nodal impulses develops, premature beats with varying coupling as in parasystole is explicable but would not seem to be physiologically normal. An irritative focus must be suspected in such cases although it might just as well be constitutional as due to a myocarditis. The relationship to the scarlet fever must be determined

separately in each case. Consideration of the clinical symptoms is thus particularly important in such questionable cases.

The following criteria have been established for the two types of nodal rhythm and their relationship with a scarlatina myocarditis:

Active Nodal Rhythms

A high frequency, one definitely higher than that observed or expected in a sinus rhythm, is regarded as evidence of an irritation, a nodal rhythm of the active type which has superseded the sinus rhythm. This criterion is indicated by A 1.

If a nodal rhythm of practically regular frequency is replaced by a sinus rhythm, following a prolonged systolic interval, it is regarded as an indication of irritative changes in the pacemaker. The activity of a physiologically normal constant pacemaker would not be expected to cease. Such a finding has been regarded as indirect evidence of an active nodal rhythm and is indicated by A 2. This is perhaps the only way in which a low frequency nodal rhythm due to pathological activity may betray itself. Premature beats are also indicative of an irritative focus.

The active nodal rhythms are to be regarded as pathological. It seems possible, however, that they, like other active heterotopia, may be found accidentally in apparently healthy individuals, and they may be independent of the scarlatina. If they were due to a myocarditis, they would probably have disappeared upon recovery. It is also possible that a physiologically normal passive pacemaker could develop a higher frequency than normal as a result of a myocarditic irritation during the illness and thus become active in character. Upon recovery , however, it might thus resume its passive character. *A nodal rhythm whose active character appeared during the illness, and in this way revealed its connection with the scarlet fever, has therefore been considered due to a myocarditis.*

Passive Nodal Rhythms

The usual thing in the transition from a sinus rhythm of diminshing frequency to a passive nodal rhythm is that the nodal rhythm is of somewhat lower frequency. However, as mentioned above it may also be of the same frequency or somewhat higher. A return to the sinus rhythm occurs when the frequency in the S-A node increases. The systolic interval at the changeover should therefore be shorter than the preceeding. A nodal rhythm is seemed to be passive in nature when it appears during a falling sinus frequency and when it has a rate lower or only slightly higher than the latter. This type of rhythm is often seen to be replaced by a sinus rhythm from which it is separated by a short systolic interval. The passive type is then classified according to the type of sinus arrhythmia.

1. If the sinus arrhythmia is of the physiologically normal type with changes due to respiration and vagal factors the passive nodal rhythm has been regarded as arising in a *physiologically normal* manner and has been indicated by *P 1*. The possibility of a myocarditis producing an irritative focus of such low frequency that it has a passive character must, however, be considered. If the nodal rhythm appeared during the illness but can not be reproduced later a connection with the scarlet fever must be suspected. An extreme vegetative lability as well as a myocarditis may, however, have been the cause. This situation does not therefore justify a diagnosis of myocarditis.

2. When the sinus arrhythmia is evidence of a sino-auricular blocking or the constancy of the nodal rhythm indicates a sinus arrest, the case has been suspected of being a *pathologically* produced passive nodal rhythm. It has been indicated by *P 2*. The nodal rhythm is physiologically normal in itself, but the basic process may, on the contrary, be pathological and the result of a myocarditis.

DISCUSSION OF THE AUTHOR'S CASES.

Table 2 summarizes the findings in the authors 43 cases of coronary sinus and A-V nodal rhythms. The material includes one case (no. 3) with a focus which seemed to arise in another region of the auricles. The cases have been arranged so that those which are active come first, followed by the doubtful ones, and then those which are probably physiologically normal.

Active or Query Active Rhythms.

Case 1. This single appearance of a high frequency A-V nodal rhythm of active type was evidently due to a myocarditis.

Case 2. A high frequency coronary sinus rhythm also appeared upon a single occasion in this case. The diagnosis of myocarditis is established by other changes in the electrocardiogram in this case, but the coronary sinus rhythm is also regarded as pathological.

Case 3. This is a case with an ectopic rhythm, atypical in appearance, with a negative P_1 and a positive P_2 and P_3 (Figure 4 a) and probably having its origin in a point in the auricle outside the A-V nodal region. It probably does not involve an intraauricular conduction disturbance as the transitions were typical of a shifting of pacemaker with sudden alterations of the P wave and a changed frequency with slight arrhythmia. Since its frequency is relatively high and it also appeared immediately after work it seems to be an active, pathological type, sign A 1. It is also possible to see that it ceases with a prolonged R-R interval (figure 4 b) criterion A 2 for the active type. It was possible to reproduce this rhythm up to 6 months following the illness but not later. It is therefore regarded as having been connected with the scarlet fever.

Case 4 was a 6 year old boy who had scarlatina without evidence of rhythm disturbances. A short time later he developed a recurrence of scarlet fever at home and returned in the desquamation stage with a relatively high-frequency coronary sinus rhythm. This did not reappear spontaneously but rather at the follow-up examination following exertion and then had a significantly higher frequency than the sinus rhythm, criterion A 1. He also demon-

TABLE 2

The Coronary Sinus and A-V Nodal Rhythm Cases

Explanation:

+ = ectopic rhythm reproduced;
(+) = other alterations in the P wave at follow-up exam.
— = ectopic rhythm not reproduced.
(—) = ecg taken only at rest.
O = follow-up examinations lacking;
D = discovered at first at follow-up examination.

Ectopic centers:

C = coronary sinus;
U = upper, and
M = middle nodal rhythm;
E = originating from another part of the auricle.
A = active,
P = passive (see further discussion in the text).
n.r. = not remarkable.
s.r. = sinus rhythm

Case No.	Record No. and series	Sex and age (yrs)	Frequency: > = higher than < = lower than = equal to sinus frequency (s.f.)	Number of occurences and time following onset of scarlet fever	Follow-up examination (after 1—3 yrs)	Other changes in the ecg.	Origin of impulse	Sign
1	3054/48-O	8	120, > s.f.	1, 12th day	—	n.r.	M	A 1
2	4179/48-D	8	110 > s.f.	1, 31st day	—	Depression of S-T	C	A 1
3	1480/49-O	5	110—80, often > s.f.	from 30th day —6 months	—	n.r.	E	A 1 + 2
4	669/49-D	6	110—100, often > s.f.	1, 2nd week	+	n.r.	C	A 1 + 2
5	3616/48-P_{po}	12	At first 100, > s.f. later < s.f.	Several times alternating occasionally with s.r.	+	Depression of S-T and T	C	At first A 1, and later P 1
6	1659/49-P_{pk}	3	U) Sometimes > s.f. C) < s.f.	Several times alternating with s.r.	+	Sinus arrest	U+C	U) first A 1, later P 1? C) first P 2, later P 1?
7	517/48-D	4	At first 100, = or > s.f., later 75, = s.f.	1, 7th week alternating with s.r.	+	Premature C beats	C	First A 1+2, later P 1

8	511/48-P_5	4	85; s.f. = 65—85	9th day and 6th week, alternating with s.r.	(+)	Premature C beats	C	A 1 + 2
9	2409/49-P_{pk}	7	60—55 < s.f.	Constant, short series	+	Premature C beats, changing coupling	C	A ?
10	4232/47-C_2	6	85, = s.f.	Only at the follow-up examination	D	Premature C beats	C	A ?
11	3570/46-P_1	10	95—100, regular s.r. irregular	1. alternating with s.r.	(—)	n.r.	C	P 1 + A 2
12	2902/48-O	10	70—100 < s.f.	Always during rest, sometimes after work	+	n.r.	U	P 1 or P 2
13	1119/48-C_2	11	70—80, = or < s.f.	Almost constant	+	n.r.	C	P 1 or P 2
14	525/48-D	16	70—80, often a little > s.f.	Constant, alternating with s.r.	—	P = 0.11 seconds	C	P 1
15	3388/47-P_2	12	Low-freq. = or > s.f.	Almost constant	+	Escaped beats	M	P 1
16	4279/47-D	8	< or = s.f.	1	+	Escaped beats	M	P 1
17	3991/46-C_1	28	< s.f.	1 of 2 ecg	+	n.r.	C	P 1
18	725/47-C_1	6	< s.f.	1 of 2 ecg	+	n.r.	C	P 1
19	951/47-C_1	35	< s.f.	Always at rest	+	n.r.	C	P 1
20	412/48-D	6	< s.f.	1	+	Transitional forms	C	P 1

Cont. next page

TABLE 2 cont.

Case No.	Record No. and series	Sex and age (yrs)	Frequency: > = higher than < = lower than = equal to sinus frequency (s.f.)	Number of occurences and time following onset of scarlet fever	Follow-up examination (after 1 —3 yrs)	Other changes in the ecg.	Origin of impulse	Sign
21	1311/48-P_5	9	< s.f.	Several times, alternating with s.r.	+	n.r.	C	P 1
22	2356/48-C_2	2	100—110, somewhat < s.f.	Several times alternating with s.r.	+	Transitional forms	C	P 1
23	3028/48-O	7	Somewhat < or = s.f.	2, alternating with s.r.	+	n.r.	C	P 1
24	3709/49-P_{pk}	7	< s.f.	Almost constant usually at rest	+	n.r.	C	P 1
25	4196/48-C_3	8	Low-frequency		+	n.r.	C	P 1
26	4725/48-P_{pk}	7	= s.r.	Several times, alternating with s.r.	+	n.r.	C	P 1
27	1246/49-D	6	Low-frequency	1	+	n.r.	C	P 1
28	1474/49-P_{po}	3	Low-frequency	1.	+	Transitional forms	C	P 1
29	3620/47-D	4	Somewhat < s.f.	2. alternating with s.r.	+	Transitional forms	C	P 1
30	4082/47-C_2	8	Somewhat > s.f. irregular s.f.	A few times, after long systolic intervals	+	n.r.	C	P 1

31	3159/48-P_{po}	5	Low-frequency = s.f.	1, only in alternate day ecg.	(+)	n.r.	C	P 1
32	3662/47-C_2	15	70 and 110, = or < s.f.	2, alternating with s.r.	—	AV block	C	P 1
33	4813/47-C_2	9	Approx. 80 = s.f.	1, 23rd day	—	n.r.	C	P 1
34	2042/48-P_2	9	65, somewhat < s.f.	1, alternating with s.r.	—	n.r.	C	P 1
35	3453/48-P_{pk}	11	Low-frequency		(—)	Transitional forms	C	P 1
36	4781/48-P_{pk}	6	= s.f.	1, after long systolic intervals	—	n.r.	U	P 1
37	1972/49-O	8	85, somewhat > s.f.	1, alternating with s.r.	—	n.r.	C	P 1
38	3443/47-P_3	11	80—85 < s.f.	2	—	n.r.	C	P 1
39	3179/48-P_{pk}	8	80, s.f. = 70	1	0	n.r.	C	P 1
40	4822/48-P_{pk}	3	Approx. 80, > s.f.	1	0	n.r.	C	P 1
41	1702/49-O	6	65—90, < s.f.	2	0	n.r.	C	P 1
42	3873/47-C_2	22	Low-frequency, = s.f.	2, alternating with s.r.	0	n.r.	U	P 1
43	49/49-P_{po}	8	Somewhat < s.f.	Only at the follow-up examination	+	A-V block	C	P 1

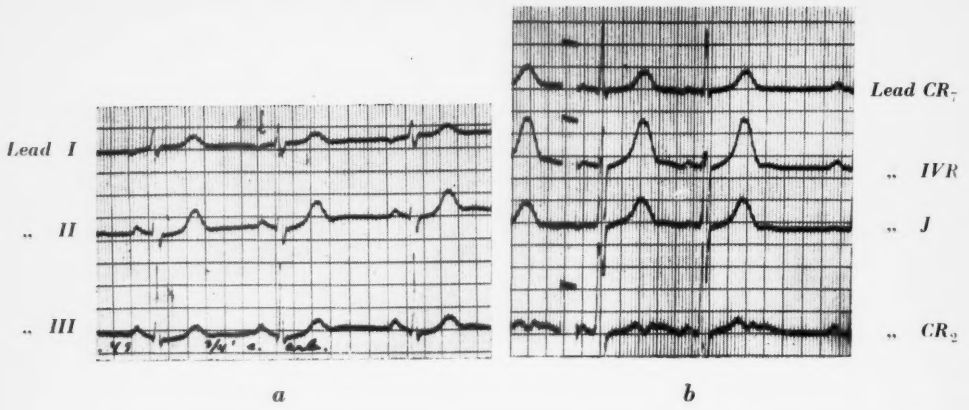


Fig. 4. *Ectopic auricular rhythm.* (Record no. 1480/49, case 3 in the "nodal" rhythm group.)

a. Limb leads immediately after work, showing P_1 negative and P_2 and P_3 positive.

b. In the precordial leads, CR_7, IVR, J and CR2, two ectopic beats are seen at the beginning with diphasic P waves. There is subsequent transition to sinus rhythm with positive P waves following a prolonged systolic interval. (Sign A 2 — see text.)

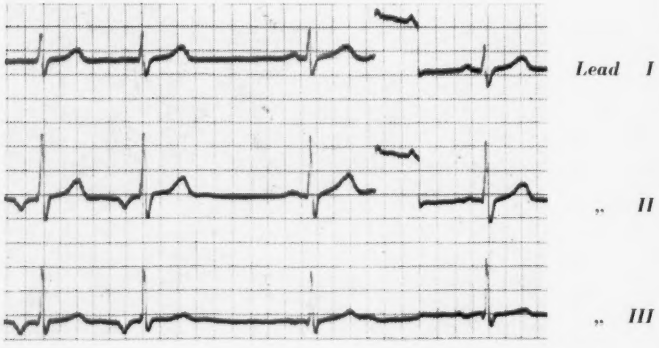
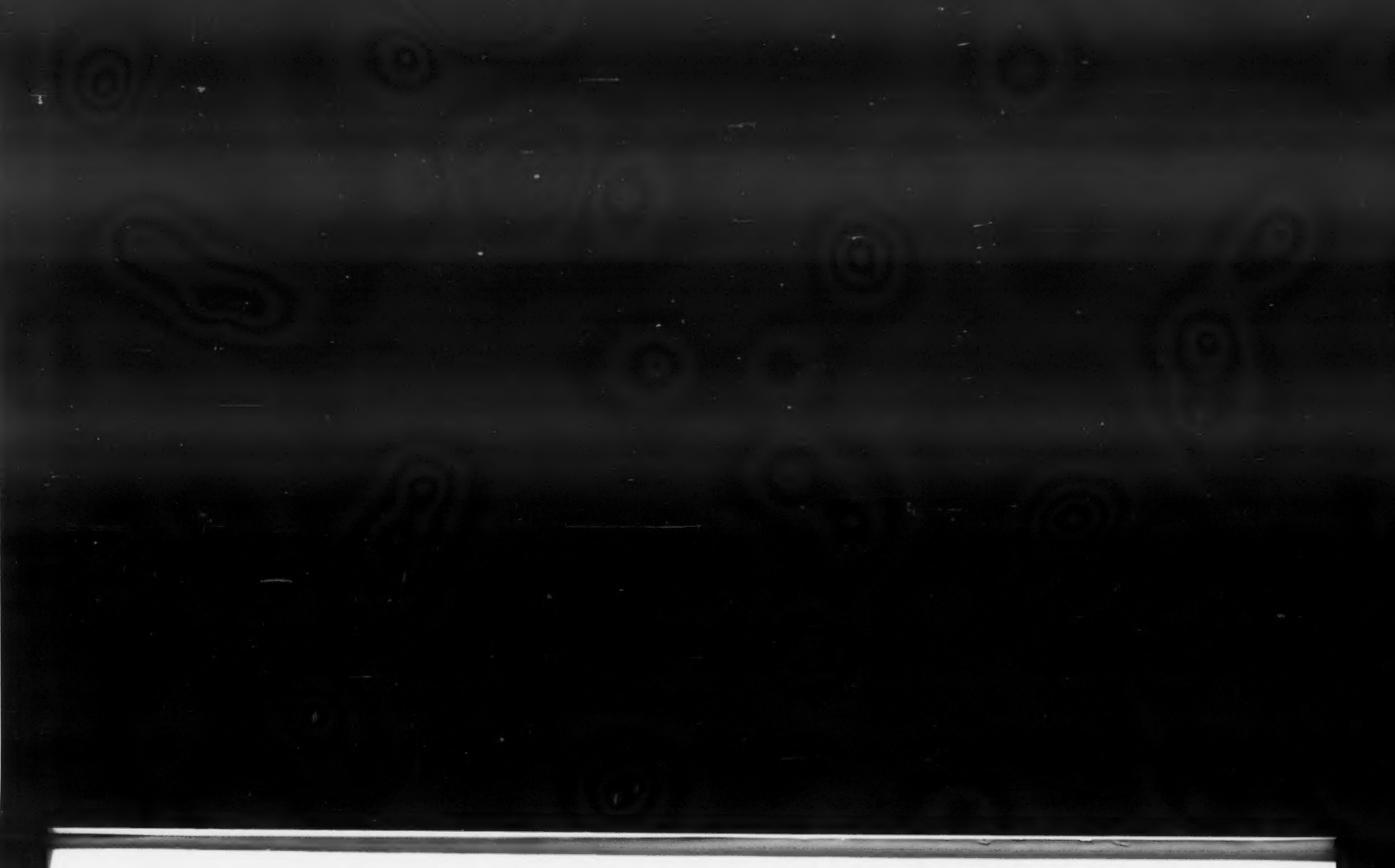


Fig. 5. *Active coronary sinus rhythm.* (Record no. 669/49, case 4.)

Limb leads 3 minutes after work. The high frequency coronary sinus rhythm in complexes one and two changes into a sinus rhythm of lower frequency (sign A 1) after a prolonged systolic interval (sign A 2).



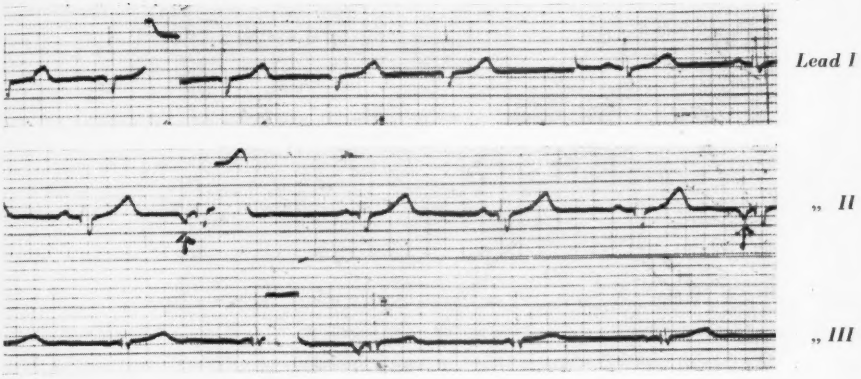


Fig. 6. *Ectopic rhythm and premature systoles from the coronary sinus area.* (Record no. 511/48, case 8. Leads I—III.)

In lead I a relatively fast ectopic rhythm with isoelectric P waves, ceasing with a prolonged systolic interval, is seen. Leads II and III show premature systoles from the same focus.

strated sign A 2 (figure 5). The high frequency nodal rhythm is seen to be replaced by a lower frequency sinus rhythm following a prolonged R-R interval. It was therefore felt that a pathological, irritative process had developed during the recurrence of the scarlet fever. However, the relation to the scarlatina is somewhat doubtful, since the high-frequency nodal rhythm was seen also at the follow-up examination. This might indicate a persistent myocardial damage. The boy had been extremely tired following the disease.

Case 5. In addition to a coronary sinus rhythm this boy demonstrated other significant signs of myocarditis with depression of the S-T segment and flattening of the T wave. His coronary sinus rhythm is coincidentally of the high-frequency active type. In contrast, later electrocardiograms at the time of follow-up examinations showed a passive type of nodal rhythm with a lower frequency than the sinus rhythm. The nodal rhythm would therefore seem to be of changing character in this case—being at first active, pathological; later passive, physiologically normal.

Case 6 has been reported earlier in detail in the chapter dealing with sinus arrest (page 37, figure 3). An active nodal rhythm seems to be illustrated in figure 3 a, and a myocarditis is regarded as probable. There was also a coronary sinus rhythm. The A-V nodal rhythm also reappeared at the follow-up examination but was then of the passive type.

Case 7 was a 4 year old girl admitted in the desquamation stage of scarlet fever with otitis media and nephritis. In the seventh week of her illness she developed an ectopic rhythm and premature systoles of the coronary sinus type which appeared several times. The frequency usually was higher than that of the S-A node, and the ectopic rhythm sometimes desisted after a prolonged R-R interval. (Criterion A 1+2.) For this reason it was felt that an ectopic focus of varying activity was present. The premature beats also indicated a focus of increased irritability. At the time of the follow-up examination, sinus rhythm prevailed both at rest and from 1—5 minutes after work. Only after deep breathing and at a relatively low frequency did a coronary sinus rhythm appear, and this was then of the passive type. The previous active nature would seem to indicate, however, that a myocarditis was present during the scarlet fever. There were no heart complaints.

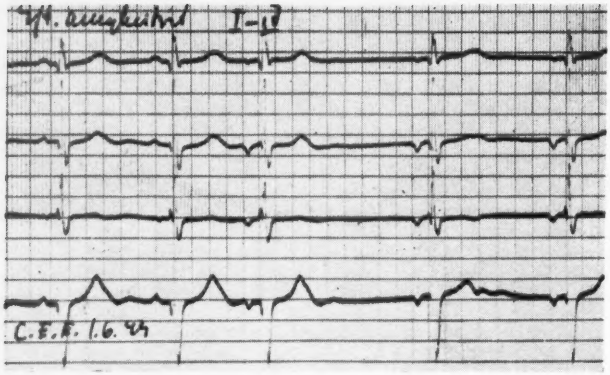


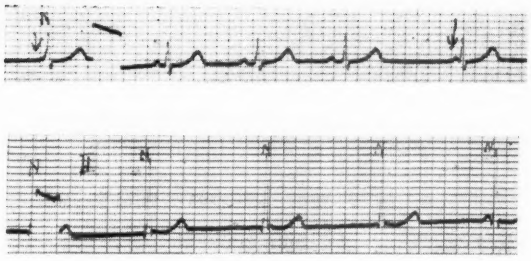
Fig. 7. *Coronary sinus rhythm with variable, low frequency* (Record no. 2409/49, case 9.)
Leads I—III and IVR following inhalation of amyl nitrite. After 2 sinus beats a series of coronary sinus beats with short coupling and of low frequency appears.

Case 8. Here a coronary sinus rhythm of somewhat higher frequency than the sinus rhythm alternated with coronary sinus beats appearing as premature systoles. The coupling varied slightly. The ectopic rhythm started with a short coupling, and this in association with the relatively high frequency indicated irritation—active character sign A 1. When the ectopic rhythm desisted (figure 6) the transition occurred with a prolonged R-R interval—the indirect sign of an active character, A 2. This case would thus seem to be pathological. The alteration could not be reproduced at later examination and has been classified as a myocarditis. No heart symptoms were present.

In case 9 the presence of the pacemaker is most frequently betrayed by premature systoles with a varying coupling. These were often followed by a short series of ectopic beats of low frequency (see figure 7 taken after the inhalation of amyl nitrite). The sino-auricular node seemed to be temporarily displaced by an ectopic pacemaker whose impulses happened to meet non-refractory muscle three times in succession. The phenomenon is suggestive of a parasystole. However, a varying interval was found between the single ectopic beats, which came in a series. Besides, the intervals between the ectopic beats have no common divisor. Therefore such a record could most suitably be classified as that due to an ectopic pacemaker of changing frequency. Despite the relatively low rate, it can be seen that an independent activity, uninfluenced by the sinus rhythm, is responsible for this finding seemingly pathological in type. Yet its persistence over a symptom-free period of 2½ years makes its connection with the scarlatina doubtful.

Case 10 was requested to appear for a follow-up examination because she had coronary sinus premature systoles on one occasion during her scarlet fever. The coupling was constant. The work electrocardiogram recorded on this occasion revealed, however, a coronary sinus rhythm. The frequency was the same as that of a sinus rhythm in a later recording. The ectopic rhythm in itself would therefore seem to be of passive type, but the earlier appearance of premature beats makes it suspicious that there was on that occasion an irritative change. There were no heart symptoms. This was regarded as an equivocal change.

In case 11 nodal rhythm of the passive type made its appearance during one of the long systolic intervals associated with sinus arrhythmia (sign P 1). The point where it stops is indicated by a pause (sign A 2). The changing activity might have its origin in some irritative focus. Nevertheless a diagnosis of myocarditis would seem to be too rash.



a. Lead I

b. Lead II

Fig. 8. *Passive ectopic rhythm and escaped beats from the A-V node. Interference and partial interference with the sinus rhythm.* (Record no. 3388/47, case 15.)

a. Lead I shows sinus arrhythmia with escaped beats in the first, second and fifth contractions. There is partial interference with the sinus rhythm which gives origin to P waves with shortened P-Q intervals in the ectopic beats. In the first beat the P wave is nearly concealed in the ventricular complex.

b. Lead II showing the nodal rhythm interfering with the sinus rhythm. In the last contraction there is partial interference with the sinus rhythm producing a superimposed P wave.

Passive Nodal Rhythms.

Cases 12 and 13 were two girls aged 10 and 11 years. The nodal rhythm in these cases seems to be of the passive type (P 1) but was unusually persistent. It was consistantly present during rest and often after work with a frequency of up to 100. On the other hand, it was always possible to bring out the sinus rhythm in some phase of the work test or with deep breathing, and it was of a higher frequency than the nodal rhythm. In these cases we would seem to be dealing with an ectopic pacemaker of unusually high-potency which competes with the sino-auricular node. The latter, however, predominates at higher frequencies. It would seem to be worth noting that both of the girls are reported to be more short of breath following scarlatina than they had been previously. This was also observed by the dancing teacher of one of the girls, who was an enthusiastic ballet dancer. The x-ray of the heart was normal. At follow-up examinations at the age of 12 both girls were unable to manage 450 Kg.M./min. and thus may have suffered from a slight reduction of their functional capacity. It might then be asked if there were not some heart damage present, in particular a lesion in the sino-auricular node with reduced irritability. The nodal rhythm would thus be of type P 2. It might then be suspected, that the heart frequency was too low and inadequate for the exertion being attempted. The functional capacity could therefore be reduced. Unfortunately we have no electrocardiogram recorded prior to the onset of the illness. If the nodal rhythm actually appeared during the scarlatina it is most probable that it was due to a myocarditis. Because of the cardiac type of symptoms, these cases were grouped under the heading of probable myocarditis with equivocal electrocardiographic changes.

Case 14, a 16 year old boy, was found to have a nodal pacemaker of the passive type most of the time during his illness. At the time of the follow-up examination, however, it did not appear although the frequency at this time was low. In addition he consistantly displayed broad P waves (0.11—0.12 sec.) but no signs of mitral valvular lesion. It might therefore be asked, whether there was not a pathological process with a delayed auricular

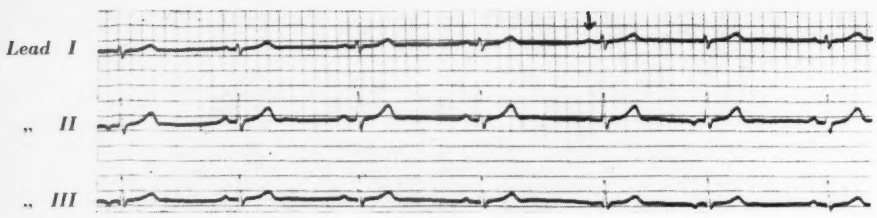


Fig. 9. *Wandering of the pacemaker between the sinus node and the coronary sinus area incident to pressure on the carotids.* (Record no. 3709/48, case 24.)
The first and sixth contractions originate in the coronary sinus region, and P_2 and P_3 are negative. A transitional form is seen at the arrow (fifth contraction) with a weakly positive P_2: a perisinus rhythm.

conduction and irritation of the pacemaker. This case has been classified as "query pathological".

Case 15 and 16 illustrate a middle nodal rhythm of passive nature occurring simultaneously with isolated ectopic systoles of the escaped beat type. A partial interference (see page 43) was often present with the result that the positive auricular wave originating in the sino-auricular node appeared with a varying interval between it and the ventricular complex stimulated by the A-V node (figures 8 a and b). It would seem that these phenomena are readily explicable as manifestations of a physiologically normal pacemaker standing in readiness. The mechanism is set in motion by the long pauses during the sinus arrhythmia and is regarded as being physiologically normal. The ectopic beats could be reproduced at a follow-up examination.

The other 27 cases, 17—43, all gave the impression of being passive. The ectopic rhythm appeared when the sinus frequency was slowed. They had a relatively low frequency which was usually lower but sometimes a little higher than the sinus rhythm. The sinus rhythm in these cases showed a respiratory arrhythmia; and, as indicated previously, in such cases an ectopic rhythm even of somewhat higher frequency than that of the S-A node may be passive. The pacemaker frequently shifted spontaneously during the recording of the electrocardiogram. In 12 patients (cases 17—28) the coronary sinus rhythm could be reproduced at follow-up examinations. In case 43 the nodal rhythm was observed only on this occasion. This supports the assumption that in these patients they were physiologically normal constitutional pacemakers. In 3 patients (cases 29—31) minor changes in the P wave appeared at the time of the follow-up examination. These were probably due to a somewhat more limited wandering of the pacemaker although of the same nature as the previous one. With more intensive stimulation the pacemaker would probably have been shifted all the way to the coronary sinus area. Such minor changes in the P waves may also be seen as transitional steps between a nodal and a sinus rhythm (figure 9).

At the follow-up examination the nodal rhythm failed to appear in 7 patients (case 32—38) but one of them was incompletely studied. In case 32

another alteration also was observed during the illness — a prolongation of the P-Q time indicating a myocarditis. The nodal rhythm, however, did not give the impression of being pathological. In case 33 it was reported that the patient developed palpitations and dyspnea immediately following the illness. It must be noted, of cource, that a myocarditis may be present although the nodal rhythm is apparently of the passive type. This must be particularly suspected in cases where the nodal rhythm cannot be reproduced following recovery. It is difficult, however, to determine whether the pacemaker appeared as a result of extreme vegetative lability or of a myocarditis. In addition, when passing cardiac symptoms (as in case 33) are met with after the scarlatina, an acute myocarditis is believed to be the probable cause. Such cases have been grouped under the heading: Probable myocarditis with equivocal electrocardiographic changes and cardiac symptoms.

As mentioned in the introduction the coronary sinus area must be expected to possess the strongest autonomic properties of the actual pacemakers and its frequency must be influenced by vegetative factors. The majority of the cases, 36, were also coronary sinus rhythms which often showed a marked respiratory arrhythmia. In four cases an upper nodal rhythm was present, but as mentioned earlier, the boundary between such a rhythm and a coronary sinus rhythm is difficult to locate. Only three cases had a rhythm originating in the middle region of the A-V node; and none were associated with the lowest part. In only one patient could the type of rhythm seen be traced to some other region of the auricle and in that case the pacemaker was pathological in type.

The frequency of coronary sinus and A-V nodal rhythm was 1.5 per cent in the whole material. The distribution of the cases with reference to sex and age (children vs. adults) as well as to different series was most nearly uniform. Nodal rhythms were observed in 1.3 per cent of those treated with penicillin, 2.0 per cent of the control cases, 1.4 per cent of the desquamating cases, and 1.7 per cent of the others.

Summary

To sum up it may be said that only 8 of these 43 cases of coronary sinus and A-V nodal rhythm seemed to indicate a scarlatina myocarditis. In another 3 cases the type of rhythm was uncertain but heart symptoms made a diagnosis of myocarditis probable. 3 rhythms were query pathological, and the other 29 were possibly physiologically normal phenomena. Of the latter there were 14 which could be said to be quite definitely constitutional in nature and could be reproduced at a follow-up examination. In another 3 cases a minor displacement of the pacemaker appeared instead. In 6 of the cases of passive nodal rhythm no shifting at all reappeared and here a connection with the scarlatina must be considered, either on the basis of myocarditis or, more probably, vegetative factors.

CHAPTER 7

Changes in the P Waves

This chapter will deal with minor variations of the P waves. These include various gradations on the positive side, or between positive and isoelectric or diphasic P waves in leads I and II, as well as the whole range from positive to negative in lead III. The variations sometimes occur spontaneously during a single recording and sometimes from one occasion to another. Broadenings and other aberrations of the P waves are also discussed.

Previous Experiences with Changes in the P Wave

Normal Material

It has been found in studies on normal subjects, especially children, that the P waves, in particular P_2, can vary considerably from one individual to another. It would also seem probable that the range of variation for each individual is rather large. Direct investigations apparently have not been made of this relationship in the case of children. However, Stewart and Manning as well as Graybiel et al. have studied the changes in the P wave in a group of normal young men. Stewart and Manning have observed these changes 12 times in their 500 case series, and they believe that they are due to respiration. Graybiel et al. found variations of the P wave with a constant P-Q time 23 times in their 1,000 cases. They state that the variations are due to a changing pacemaker in and around the sino-auricular node. To quote further from this author: "This unimportant form of arrhythmia has no pathological significance". A corresponding classification is found in the Nomenclature 1945, where small changes in the P wave are put under the heading of wandering pacemaker within the sino-auricular node.

Pathological Material

The interpretation of changes in the P wave in possible cases of heart disease depends, of course, on the supposed pathogenesis. As in the case of nodal rhythm changes in the P wave have often been recorded as pathological, as by Lukomski (1932) in cases of rheumatic fever. Spühler (1939) and Marcel (1939) interpret alterations in the P wave, at a constant frequency, as sino-auricular disturbances. Wendkos and Noll (1944), on the other hand,

state that changes in P_2 and P_3 are due to a wandering pacemaker and are doubtful evidence of myocarditis. A changing P_1, however, is recorded as significant and evidence of an intra-auricular disturbance. Sampson (1946) believes it is more likely that the P wave changes depend on altered conduction rather than on a wandering pacemaker, and he regards them as significant since they parallel the clinical course. Filberbaum (1946) and Sokolow (1948) regard them as the result of shifting auricular pacemaker and apparently ascribe a pathological significance to them. Nadrai (1941) states that P changes are signs of a changed origin of the stimulating impulse. According to him they may be due to both vegetative lability and myocarditis as for example in scarlatina and rheumatic fever. In their studies of scarlatina, Spang and Welsch (1947) have reported changes in the form of the P wave as pathological and evidence of auricular myocarditis. Klemola (1942) has taken particular interest in these P alterations in his studies of convalescents from infectious diseases, particularly diphteria. He usually classifies them, as does Korth (1941), as "vagische P" of functional nature and without association with auricular damage. When P changes persist as an ectopic rhythm over a longer series of contractions, Klemola regards them as evidence of increased vegetative lability and, in the case of diphteria, possibly also of increased irritability in an ectopic focus.

The Bases of Classification and Interpretation in this Study

Before P variations can be interpreted with regard to significance as to myocarditis or not it would seem to be necessary to classify them according to their genesis. Alterations of the P wave can be considered as occurring in any of the 4 following ways as a result of:

1) Vegetative-hemodynamic alterations, causing a change in the functional state of the heart.
2) Vegetative-hemodynamic alterations causing displacement of the pacemaker ("perisinus rhythm").
3) Auricular myocarditis with coincident conduction disturbances.
4) Auricular myocarditis with displacement of the pacemaker.

Discussion of the Various Types

1. Vegetative-hemodynamic alterations directly producing changes

The most natural and probably commonest cause of changes in the P wave are vegetative and hemodynamic alterations during established sinus rhythm. They result in variations of the functional state of the heart and thereby in changes of the electrocardiogram. They are reflected in the frequency, and in the case of pure vegetative changes the T waves also shift in the opposite direction from the P waves (see Nordenfelt 1941 among others). During work the relationship is more complicated. This group of changes lacks independent significance and should seldom cause difficulties in diagnosis.

2. Vegetative-hemodynamic alterations causing shift of the pacemaker

Changes in the P waves similar to those above, in which P_2 is obviously lowered or even becomes isoelectric and P_3 is lowered and often becomes negative, may also result from a wandering of the pacemaker either within the S-A node, which is a relatively large area, or to a adjacent portion of the auricle. For these, I should like to propose the term "perisinus rhythms". Their frequency is ordinarily somewhat lower than that of the S-A node. Levine et al. (1949) with the help of heart catheterization, have succeeded in tracing the origin of such a perisinus rhythm to a region half-way down the auricle, viewed in relation to the movements of the catheter.

It is thinkable that a coronary sinus rhythm may also be accompanied by an isoelectric P_2. It is almost impossible to make an exact differentiation between these various ectopic rhythms, and there is scarely any practical reason for doing so since they are closely related. The border-line in this work has been so arranged that negative P_2 is regarded as indicative of a coronary sinus rhythm while isoelectric and disphasic P_2 are taken as meaning a perisinus rhythm.

A sudden change in the P wave is characteristic. Pronounced differences in frequency are not necessarily found between these closely adjacent centers, and the P-Q time is slightly shortened or unchanged. Vagal stimulation often associated by respiration may provide the impulse for such a shift of the pacemaker within the S-A node or its vicinity as well as to the A-V node (see further page 43). The P waves of these perisinus rhythms well deserve the name "vagische P" (Korth). As early as 1915 Wilson described this type of respiratory changes in the pacemaker as did Stewart and Manning in 1944. Such variations could be reproduced by vagus stimulation.

The differentiation between these two types of changes in the P wave as a result of vegetative influencies is of more theoretical than practical interest. As a matter of fact both are entirely normal manifestations. The frequency changes are similar, with higher P waves at higher frequencies. When a lowering of the frequency occurs, the arrhythmia, however, usually becomes less obvious if there is a perisinus rhythm, but it is more noticeable if the sinus rhythm has not been interrupted. The differentiation is easier when a series of electrocardiograms is available. On the whole it may be said that a direct vegetative influence has a more marked effect on the frequency and in relation to this a more gradual and less noticeable effect on the P wave than has a vegetatively caused wandering of the impulse center. Even a small change in the autonomic tone with no or only slight alterations in other aspects, for example on the frequency and the T wave, can produce a marked and often abrupt change in the P wave. Transitional forms of the P wave may appear, however, also during a wandering of the pacemaker to the perisinus area, a similar phenomenon to that described earlier in A-V rhythm. Spontaneous

changes in the P wave during rest are probably the result of a shift of the pacemaker, since vegetative alterations during rest are seldom of such magnitude that they would directly produce obvious alterations in the P wave.

3. Auricular myocarditis with conduction disturbances

If the P waves change when the frequency and P-Q time are constant, altered intra-auricular conduction must be suspected as noted by Scherf and Schokhoff (1926), Rothberger and Scherf (1926) and Scherf and Boyde (1945), in particular. The P-Q time, however, may also be unchanged with small shifts of the pacemaker particularly as the delay in the conduction of the stimulus between auricle and ventricle mainly occurs in the region where the auricular and ventricular parts of the A-V node meet, and the change in frequency may also be insignificant. Such a comparison is less valid when the variation is from one occasion to another but can be made when the variation has occurred during the course of a single recording. A closer analysis of P wave variations is really possible only in these cases. Intra-auricular conduction disturbances might be suspected otherwise when P wave forms appear which differ from those usually found in perisinus rhythm. As with other changes due to myocarditis, these variations in the P wave should disappear after recovery from the myocarditis.

4. Auricular myocarditis with shift of the pacemaker

A wandering of the pacemaker to the vicinity of the S-A node, a "perisinus rhythm", may also be caused by an irritative focus, a myocarditis. If such an ectopic rhythm is of high-frequency type it should be possible, as in the case of nodal rhythm, to differentiate it from a physiologically normal perisinus rhythm. In the case of myocarditis the rhythm is not likely to be reproducible in its original form after recovery.

Differential Diagnosis of the Various Types

Studies of the transitions between different types of P waves as well as attempts to reproduce the earlier picture at later follow-up examinations are of great importance. The electrocardiogram is taken at this time with deep breathing, vagus stimulation, and after work. If the P wave changes reappear they probably were physiologically normal in nature. The transitions show whether their character is that of a changing impulse center or not. If the variations in the P wave cannot be reproduced a myocarditis rather than a physiologically normal perisinus rhythm is suggested. If a recording showing the transition from one type of P wave to another, is not available, the differentiation between an ectopic pacemaker and disturbed intra-auricular conduction will be difficult. This can then be done only if an intra-auricular disturbance has caused an aberrent P wave or if an active high-frequency ectopic pacemaker is present. Persistent auricular damage can be distinguished from the normal causes of P wave changes only on the same presumptions.

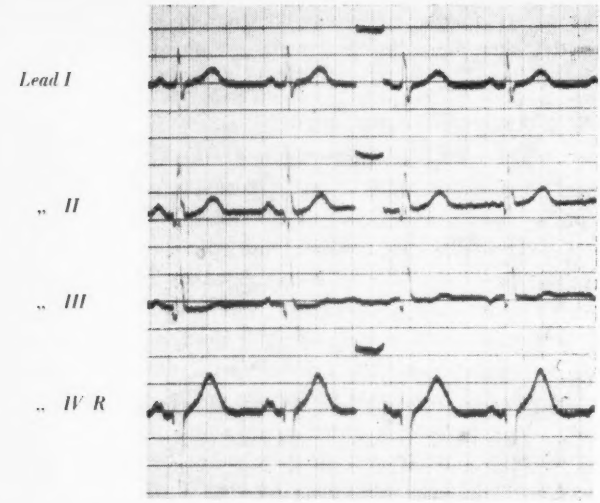


Fig. 10. *Spontaneous shift from sinus rhythm to perisinus rhythm.*

Both of the first contractions originate from the sinus node and show positive P waves in all leads. In the two later contractions P_1 and P_4 are positive, P_2 diphasic, and P_3 is negative. The transition follows a slightly prolonged systolic interval, and the perisinus rhythm has a somewhat higher frequency than the sinus rhythm.

Cases in the Present Study

Changes in the P wave often occurred in this scarlet fever series. In 45 cases variations in the P wave were observed which could not be explained by direct vegetative alterations. In addition, in 9 patients with coronary sinus rhythm smaller changes of the P waves were seen as transitional forms. In 42 cases P_2 and P_3 varied while P_1 remained almost constant. P_2 has ranged from highly to weakly positive 11 times; from positive to isoelectric 20 times; and from positive to diphasic 11 times. Simultaneously P_3 has varied between various grades of positive or negative. P_1 has varied only once (record no. 1518/49) from positive to diphasic and simultaneously P_2 and P_4 became diphasic.

Only two cases of possible intra-auricular conduction disturbance were observed and are to be discussed under "Follow-up examinations". The transitions occurred in all other 43 cases as would be expected with a wandering of the pacemaker also in the single case with changes in P_1. When the P waves were lowered the frequency diminished and became more regular than was the case with the higher P waves. At times the change occurred so abruptly that it clearly showed that something other than a hemodynamic change had taken place. Figure 10 shows such a sudden change in the P wave simultaneous with a slight increase in frequency. The natural explanation of this would

seem to be a wandering pacemaker. Sometimes the change in the P wave occurred gradually and a slight shortening of P-Q time also developed in some cases. It was possible to see minor changes in the P wave in the same patient during one recording, while during another a coronary sinus rhythm appeared under similar variations in frequency.

It would thus seem likely that the changes in the P wave observed in these cases were in the nature of a wandering pacemaker or perisinus rhythm. Frequently the change was clearly observed to occur with the various phases of respiration. The physiologically normal nature of the changes was repeatedly demonstrated at the follow-up examinations of children who had not shown such changes previously but were checked for other reasons. Variations in the P wave developed in a number of these cases when deep breathing or eyeball pressure were tried.

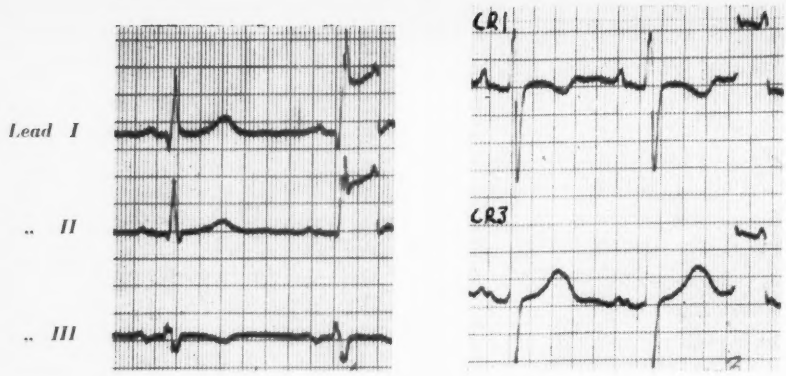
Follow-up Examinations

Follow-up examinations were made 1—3 years later upon 12 children, who had variations in the P wave during the illness. In 9 of them the alterations could be reproduced indicating that they were physiologically normal phenomena. Of these 9, 4 had had other changes in the electrocardiogram suggestive of myocarditis. In contrast to the P wave changes these could not be reproduced.

In the 3 remaining cases the P wave variations did not reappear. One of these was a 6 year old boy (record no. 1692/49) where P_2 had changed from positive to diphasic. This variation was considered to be rather suggestive since the frequency was relatively high. He had been fretful and tired following scarlet fever but there were no heart complaints. This child was very much afraid at the follow-up examination, and it is possible that the associated sympathetic stimulation prevented the appearance of the pacemaker in spite of deep breathing. The case has been classified as equivocal.

The two other cases were both 10-year old girls. One of them (record no. 2251/49) had in most of her electrocardiograms, including those taken at the follow-up examination, a P wave which was 0.10 sec. and a P-Q time of 0.14 sec. In the third electrocardiogram P was broadened, 0.14 sec., partly split and the P-Q time varied between 0.19 and 0.22 sec. (Because of the concomitant P changes the case has not been counted among those of A-V block). This variation did not reappear during the follow-up examination and there seems to have been an intra-auricular, possibly, in addition, an atrioventricular conduction defect. This change is grouped under the cases of myocarditis.

The other girl (record no. 2205/48) manifested considerable variations in the P-Q time (0.11—0.16 sec.) during the course of scarlet fever. At the same time the P wave varied so markedly that it was felt that a changing pacemaker was more likely the cause than a disturbance of A-V conduction. Two years later the P-Q time variations reappeared but the P wave remained constant. For this reason the earlier changes of the P wave are suspected of being



a *b*

Fig. 11. *Constant abnormality of the P waves — probably due to asynchronism of the auricles.* (Record no. 1912/48.)

a. Limb leads showing P_2 with a double apex and P_3 to be diphasic.

b. A thin, high P wave is seen in lead CR_1, corresponding to the right auricle, while in CR_3, corresponding to the left auricle, the apex of the P wave is doubled and the second apex is seen to be 0.04 seconds later than the right auricular wave and the first apex.

pathological. In this case an intra-auricular disturbance was possibly present, but the author does not feel able at the present time to make a diagnosis of myocarditis on the basis of this change alone.

Summary

A total of 45 cases with minor variations in the P wave have been observed. In the majority the changes seem to have been caused by physiologically normal wanderings of the pacemaker, perisinus rhythms. In one case, however, the cause was obviously an intra-auricular conduction disturbance.

Further Aberrations of the P Wave

In one patient, a 7 year old boy (record no. 1912/48), a constant abnormality of P_2 was found consisting of splintering with 2 peaks. P_1 was positive. In addition to scarlet fever he had had repeated attacks of bronchitis. At the follow-up examination 2 years later the P wave was unchanged (figure 11). The precordial leads revealed an early, high and narrow P wave over the right auricle in lead CR_1. Over the left auricle, lead CR_3, the second peak of the P wave appeared 0.03 to 0.04 seconds after the first one (figure 11 b). An abnormally long time difference between the auricular contractions seems to be present and probably cause the splintering of P_2. According to Nadrai such asynchronism of the auricles may be observed with myocardial damage or with vagotonia. In this boy it seems in any case unlikely that the condition is related to the scarlatina, since it remained unchanged during a long observation.

CHAPTER 8

Ectopic Systoles

(Premature systoles, parasystoles, and escaped beats)

It would seem resonable to group premature systoles, parasystoles, and escaped beats in one chapter under the term "ectopic systoles". They have in common their ectopic origin and the fact that they interrupt the basic rhythm, and they may be confused with one another. In the case of premature systoles and parasystoles the differentiation is possible only in long recordings or on repeated examination. Typical features of these three forms of ectopic systoles are employed in making the differential diagnosis, and this forms a suitable basis for their classification.

Premature Systoles (extrasystoles)

Definition and Diagnosis

The premature appearance prior to the expected normal systole is the first typical characteristic of the premature systole. This distinguishes them from escaped beats which manifest themselves in the long pauses during sinus arrhythmia or sinus arrest. Their second characteristic is that of dependence upon the preceeding systole manifested in the fixed coupling. This differentiates them from parasystoles which have an independent rhythm unaffected by the sinus impulses and a variable coupling.

Appearance and Significance

Premature systoles are undoubtedly abnormal in character, and according to Wenckebach and Winterberg evidence of active heterotopia. They show an increased irritability in a particular focus and seem to appear without purpose in contrast to escaped beats. The contraction which they cause is weak and sometimes incapable of opening the semilunar valves and therefore must be regarded as having no useful purpose and interfering with the function of the heart. In spite of this it has been possible on the basis of long practical experience to reject a pathological significance for them in a large number of cases. They have over a long period of time been observed in people who have apparently had otherwise normal hearts. In these cases they have appeared so seldom that the heart function as a whole has not been disturbed. These

more constitutional than pathological extrasystoles also have a tendency to disappear with work and during acute illnesses when they might otherwise be more deleterious.

Premature systoles are not an unusual finding in normal subjects. Graybiel et al. found them in 1.5 per cent of grown men and Stewart and Manning in 2.4 per cent. Among 3629 soldiers, subjected to the stress of active service, Nathan (1949) found extrasystoles in as many as 251 cases. In children Lyon and Rauh, by auscultation, have found them more often in cases of heart disease (4.3 per cent) than normals (2.2 per cent). In Landtman's monograph on heart arrhythmias in children there are 86 cases corresponding to 1.5 per cent of the patients, or 1 per cent of all the electrocardiograms. In his study extrasystoles, in particular the supraventricular types, were commoner in patients with heart disease than others. According to tabularized follow-up studies it would seem that premature beats are more likely to disappear in cases of acute heart disease than in those without heart disease. This must be regarded as indicating that in the former cases they were connected with the illness. For that reason one should be suspicious of premature systoles in the presence of potential heart disease as for example in scarlet fever.

In the literature dealing with rheumatic diseases and scarlet fever premature systoles have frequently been associated with the disease even if they were not put directly under the heading of myocarditis (Blumberger, Crossfield, Cohn, Drawe, v. Kiss, Orgain, Pardee, Roelsen, Wickström). On the other hand, Blackman and Sokolow do not consider them at all since they are often observed in healthy subjects, while Wendkos states that they are a doubtful change. Spang and Welsch claim that they have a variable significance, and like Filberbaum et al., they regard the supraventricular-nodal ones as having a more serious implication.

These differing views merely indicate that the significance of premature beats must be determined from case to case as Nadrai has stated. It is a difficult and often impossible task to differentiate the extrasystoles due to a potential heart disease from those due to constitutional factors or old lesions. The presence of different foci and an increase or appearance after work are considered to be direct evidence of a pathological process as are runs of systoles. In this connection, however, it must be kept in mind that in all probability many extrasystoles which do not meet these criteria are pathological. If at all possible their significance can be appreciated only after long observation and careful consideration of the course taken by the disease. On the other hand, it is possible to dispute the drawing of the border line so strictly with the appearance of more than one focus. Is it not possible that several foci may exist on a constitutional basis? From a practical standpoint however, the boundary line is worthwhile since the chances of disturbed heart function are greater in the presence of several foci. An increase in premature beats during or immediately after work is also a characteristic of great practical significance

since this could decrease the individual's physical capacity. If a focus has such an effect it thus acquires a pathological significance, although it does not necessarily mean that an active myocarditis is the cause.

The Author's Cases

Premature beats have been found in 31 of the patients in this study, corresponding to 1.1 per cent.

Some relevant facts regarding these patients are given in table 3, including the origin, other changes in the electrocardiogram, and the findings at follow-up examination. In three cases the premature systoles were observed only on that occasion.

Premature beats appeared in 5 of the 129 patients who were found to have other changes in the electrocardiogram interpreted as indicating myocarditis. (The 4 patients in which premature beats as well as a coronary sinus rhythm developed from the same focus during the illness have then not been included). This figure corresponds to 3.9 per cent in contrast to 0.45 per cent (22 cases) of the 2698 patients without definite signs of myocarditis. They were thus more common in the myocarditis cases than in the rest of the material, but the difference has not been established statistically. In addition the premature beats were of such character that they had to be regarded as pathological in 4 of the 5 myocarditis cases though the cause of the extra beats was obviously the scarlatina myocarditis in only one case. Further information regarding the 4 patients are offered below. The premature beats had pathological features in a single patient with an otherwise normal electrocardiogram but their connection with the scarlet fever was not obvious. The diagnosis of myocarditis has thus never been based entirely on the presence of premature beats.

Case 1 developed a recurrent hemolytic infection with synovitis and first degree A-V block in the sixth week of illness. After four more weeks, premature beats appeared, originating sometimes from an auricular and sometimes from a ventricular focus. These extra beats have never reappeared and seem to have been definitely connected with a myocarditis.

Case 2 had a transient S-T and T depression during the first month of the illness. Furthermore, frequent auricular premature beats were observed during the entire 20 months of observations. They often increased after exertion thus seeming to be of pathological nature. Figures 12 a, b, and c illustrate how also in the premature beats the terminal deflection is affected by the myocarditis. It also shows how the varying degree of restitution, as indicated by the time relationship of the T wave to the premature beat affects the degree of aberration. Figure 12 a is from the early part of the illness during a myocarditis. The S-T segment and the T waves in the ordinary beats are depressed in leads II and III. The T waves are seen to be deeply negative after the premature beats. Curves 12 b and c are taken after recovery. The S-T segment and T waves are normal in the normotop beats, while a slight rounded depression of the S-T segment is seen after the auricular beats. In the ectopic beats the ventricular complex is of somewhat different appearance in b and c. The varying degree of aberration probably seems to depend upon how close the premature beat follows repolarization of the ventricle. The coupling is constant, but the Q-T time, which represents the period of repolarization, varies in the preceeding normal beats. The

TABLE 3

The Premature Systoles

A = supraventricular (auricular and nodal)
+ = premature beat reproduced
(+) = coronary sinus rhythm reproduced
— = premature beats not reproduced
V = ventricular focus (preceding figure indicates number of foci)
O = follow-up examination lacking
Myoc. = myocarditis.

Case No.	Record No. and series	Origin	Occurrences	Other changes in the electrocardiogram. Remarks.	Follow-up examination
1	1042/48-P_5	1 A. 1 V	Once	Myoc., A-V block	—
2	4417/48-C_3	1 A	Constant	» , S-T and T depressions	+
3	3616/47-D	1 V	After work*)	Myoc., T_1 and T_2 negative.	+*)
4	4547/48-O	1 A	Several times, also at high frequency	Myoc., T_2 isoelec.	+
5	1858/49-O	1 V	Once	Myoc., S-T and T depressions	—
6	2409/49-P_{pk}	1 A	See nodal rhythms	Coronary sinus rhythm.	+, (+)
7	517/48-D	1 A	»	»	(+)
8	511/48-P_5	1 A	»	»	—
9	4232/47-C_2	1 A	Once	» *)	(+)
10	359/47-P_1	1 V	Constant bigeminy	Old lesion.	0
11	4113/46-C_1	1 V	In both ecg.	Varying coupling.	0
12	2283/49-D	1 V	Several times	Varying coupling. Parasystole?	+
13	991/48-P_2	1 V	Twice	Superimposed P-waves	0
14	973/48-C_2	1 V	Once	P-Q = 0,06 sec. Pre-excitation?	—
15	1372/49-P_{po}	1 A	Once	Short of breath	—
16	3915/47-P_5	1 V	Once	—	—
17	2142/47-P_1	1 V	Twice	—	0
18	4887/48-D	1 V	Several times	—	+
19	915/49-I	1 A	Twice	—	—
20	4108/47-P_5	1 A	Several times	Escaped beats from the same focus.	—
21	4565/48-I	1 A	Once	—	0
22	3934/48-P_{pk}	1 A	Once	—	—
23	3605/47-D	1 A	Twice	—	0
24	895/48-P_5	1 V	Once	—	0
25	3538/47-O	1 V	Once	—	0
26	3923/47-P_5	1 V	Several times	—	0
27	4845/48-I	1 A	Once	Parasystole.	—
28	4340/48-I	1 V	Once	—	0
29	151/48-D	1 V	Once	—	—
30	4279/47-D	1 V	Once*)	A-V nodal escaped beats.	+*)
31	4196/48-C_3	1 V	Once*)	Coronary sinus rhythm.	+*)

*) Seen only at the time of the follow-up examination.

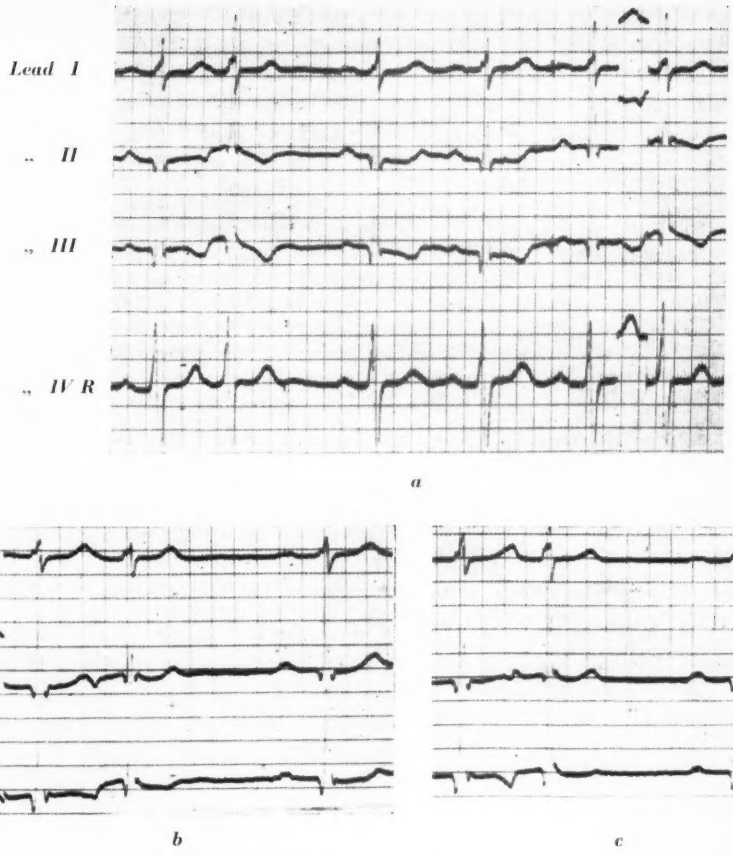


Fig. 12. *Auricular premature beats during myocarditis and after recovery with varying degree of aberration.* (Record no. 4417/48.)

a. In this recording showing the limb leads and IVR during the first part of the illness there is lowering of the S-T segment and the T wave in the normal ventricular contractions. This is accentuated in the premature auricular contractions — second and sixth in the curve.

b. and c. show the limb leads at the follow-up examination after recovery. The closer the premature beats are to the preceding T wave the larger the intraventricular aberration is. There is an earlier appearance and more marked aberration in c. than in b.

negative P wave of the auricular beat, which is seen in the distortion of the preceeding T, falls in the last portion of the T wave in figure b and in the first portion in c. As a result the aberration is more pronounced in the latter curve.

This patient, a 10 year old boy, has resumed gymnastics and sports following his scarlet fever and reports no associated difficulties. However, at the follow-up examination at the

age of 12 years he could not manage 450 Kg.M./min. It therefore seems that he suffers from a slight reduction of capacity. Since he was not examined prior to scarlet fever it is not possible to say whether there is an etiological relationship between the scarlet fever and the premature beats. It is not impossible that they were present prior to the illness and of constitutional nature, but in any case they seem to be pathological so far as the frequency is concerned.

The third patient was a 40 year old man in the desquamating stage of scarlet fever who showed negative T_1 and T_2 in the second week of illness. Subsequently the electrocardiogram became normal. At a follow-up examination after 3 years he was able to do 900 Kg.M./min. in the function test. The resting electrocardiogram was normal, but extrasystoles from a ventricular focus appeared after work. In addition the T waves in the normal complexes immediately after the premature beats were flattened. According to Scherf (1944) this favours heart disease. The primary electrocardiographic change has disappeared, but there is still the question of whether the premature systole may not be residual of the scarlet fever myocarditis.

The fourth case was that of a 7-year old girl. She had, in addition, a flattening of T_2 but as the frequency was high, a condition of increased sympatheticonia was suggested. Many of her electrocardiograms showed auricular extrasystoles, accompanied even by tachycardia. All this pointed to a pathological cause. She was much fatigued and readily breathless following the disease. Therefore, a myocarditis seemed probable. Whether her premature beats had any connection with the scarlatina is, however, questionable, as they appeared also after recovery. This case has been grouped under "Probable myocarditis with heart symptoms".

Among the patients with either otherwise normal electrocardiograms or doubtful changes was a 15 year old girl (case 10) with a constant ventricular bigeminy. This had, however, been observed some years earlier after an upper respiratory infection. In none of the other cases were the premature beats pathological in character. The diagnosis of myocarditis has thus never been based on the presence of premature beats alone.

The coupling with three exceptions (case 6, 11 and 12) was constant. One of them, case 6, had in addition coronary sinus rhythm and is discussed in chapter 6. Case 11 was only investigated twice, and just short strips of the record being available. An analysis of the intervals, which perhaps could have revealed a parasystole, was therefore impossible.

The third case (record no. 2283/49) was a 7 year old boy who was admitted in the desquamating stage with acute nephritis and acute cervical lymphadenitis. Most of his electrocardiograms taken over a period of 1 year's observation have shown ectopic ventricular beats from the same focus. The coupling was constant in some electrocardiograms; while in others it involved differences of as much as 0.44 to 0.62 sec. and 0.39 to 0.61 sec., thus differing from the usual condition with premature beats. However, it was not possible to find a common divisor for the intervals between the extra beats to indicate parasystole. The sinus rhythm was practically regular during the same period, and for this reason it may not be assumed that a changing vegetative factor was responsible for the changes in the interval of the ectopic beats. It was also impossible to find a factor such as the distance from the previous premature

beat, which could cause the variation in the coupling. This rhythm disturbance is difficult to classify. There would seem to be an "automatic" focus with a variable block which occasionally gives origin to premature systoles. As a rule the ectopic beats increase following exertion and may then occur after every or every second beat. In spite of this the patient had no subjective complaints and was able to perform 300 Kg.M./min. which is probably satisfactory for the age. The frequent extra beats, increasing after work, justify regarding the phenomenon as pathological, but it has not been possible to definitely establish that the cause was a scarlatina myocarditis, especially since nothing is known regarding the patient's electrocardiogram before the illness.

The ectopic beats were, as a rule, distinctly premature. In case 20 there were seen, apart from auricular premature contractions, escaped beats from the same focus. In two cases the extra beat came so late that it wholly or partly merged with the P wave. In one of them, case 13, an ectopic ventricular beat was seen superimposed on the P wave. The sinus frequency was almost regular, being about 100 per minute. In case 14, on one single occasion with sinus tachycardia, a complex was noticed, which had a shortened P-Q time and broadened QRS. The P-J time was insignificantly shortened in relation to the normal systoles. Here the possibility of a single pre-excitation beat has to be considered. The change was not reproducible at follow-up examination. This prevented closer study of it, and there was no possibility of making the differential diagnosis between premature beat and pre-excitation, which are commonly regarded as essentially different. Nevertheless, some voice the opinion that pre-excitation is a type of late premature beat. (Kossmann et al. 1950). In any case, it would seem most generally acceptable to group this beat along with the extrasystoles.

Eleven of the patients with premature beats not characterized by any pathological features were seen in follow-up examinations. At this time electrocardiograms were taken after work and sometimes during deep breathing as well as in rest. The premature beats were reproduced in only 1 case — possible evidence that they were related to the illness. On the other hand this may have been due to the fact that the electrocardiogram was recorded a number of times during the illness thus offering a greater chance of recording extra beats while the follow-up examination involved recordings made only on one occasion. One of these patients had, however, been rather dyspnoic a short time following the disease (case 15) and is grouped under "Probable myocarditis with heart symptoms". In two patients who were called to follow-up examination because of other rhythm disturbances ventricular premature beats were observed only on this occasion.

In regard to the origin of the premature beats, 14 were supraventricular and 18 ventricular. They were observed in 6 of the 279 adult patients (2.2 per cent) and 25 of the 2552 children (1.0 per cent). In the various treatment

groups the frequency among the penicillin cases was 0.9 per cent, among the control cases 0.9 per cent, and among the desquamating cases 1.8 per cent. None of these differences is significant.

In summary it may be said that premature systoles did not appear oftener in these scarlatina patients than in healthy individuals. The premature beats were of a definitely pathological character in only 5 out of 31 cases, but only once they were undoubtly correlated to the scarlet fever. It is possible that the premature beats in still other cases, although they showed no pathological characteristics, were due to the scarlatina because they were seldom reproducible at follow-up examination.

Parasystoles

Definition and Properties

Parasystole is a type of "para-arrhythmia" — an autonomic, low-frequency center which emits impulses independently of the sino-auricular node's activity, which is thus interfered with. In accordance with the usual rule this focus ordinarily has a distal position in the ventricle. Its existence depends upon a "protective block" which insulates it from the normal higher frequency sinus impulses (Kaufmann and Rothberger). If it were not for this, these low frequency ectopic impulses would never have time to develop between the regular contraction waves anyway. This gives the finding its special character and determines its typical appearance with single beats and varying coupling. Other ectopic rhythms, such as the A-V nodal and coronary sinus varieties, may also at times possess a parasystolic character. As they usually have a rather high frequency and so appear as ectopic rhythms, they are delt with in an earlier chapter. It therefore seems justifiable here to describe only ventricular pacemakers and no other types as parasystole in a limited sense.

The "protective block" may possibly be disturbed by digitalis for example, subsequent to which parasystole was observed to be replaced by premature systoles with a fixed coupling (Scherf and Schott). The ectopic systole then retained its characteristic appearance which is due to its position in the ventricle, but its character was altered. The independent stimulation had ceased, and the ectopic beats were now actually dependent on the normal stimulation.

A parasystole manifests itself as an ectopic beat each time the impulse arrives when the ventricle is not in its refractory period (Singer and Winterberg). For this reason the coupling varies, and in long recordings the shortest corresponds to the refractory period. The interval between impulses from the independent center is the common divisor of the intervals between the ectopic beats. On this basis it is possible to calculate the appearance of the ectopic beats to within 0.02 to 0.05 sec. It is possible that the frequency might be influenced by vegetative factors to a certain degree. Some authors (Kaufmann

and Rothberger) have supposed an "exit block" as an explanation of the fact that at a given frequency the focus may occasionally produce beats at longer intervals than calculated.

Partial Interference and "Mixed" Systoles

When the stimulus comes late in diastole so called partial interference between the sinus rhythm and the parasystole results. The normal P wave wholly or partially coincides with the parasystole. Such a combination resembles an escaped beat. When stimuli from the S-A node and the ectopic center reach the ventricles at the same time, these are stimulated by both impulses and mixed systoles result.

Diagnosis

The diagnosis demands long recordings with a number of extra beats included making it possible to analyze the interval. Single extra beats can not be distinguished from premature systoles and escaped beats. Many so-called premature systoles are probably parasystoles in reality, especially since the same coupling may appear some times by change. Parasystoles must always be suspected in the presence of a variation in the coupling, and the diagnosis is verified if a regular basic frequency can be established for the ectopic beats.

Occurrence and Significance

Parasystoles seem to be less common than premature beats. According to Faltitschek and Scherf (cit. Scherf and Boyde 1945) all cases described up to that time were in patients with definite or suspicious heart disease. So far as is known no cases have been previously described in scarlet fever. Because of the presence of an active ectopic focus with a "protective block" they are undoubtly abnormal manifestations, but like premature systoles they may result from constitutional factors which has been pointed out in a recent paper by Sherf and Boyde 1950. Therefore a parasystole should not be accepted as evidence of heart disease without further consideration. In any case they should not be accepted as evidence of myocarditis even if they happen to be observed during scarlatina.

The Author's Cases

There are 5 cases of parasystoles included in this study.

Case 1 (record no. 1637/48) was a 5 year old girl who was found to have ventricular systoles, arising from the same focus, in the routine electrocardiograms taken on the ninth, twentyninth, and forty-sixth days of illness. Their coupling varied between 0.30 and 0.90 sec., and they were sometimes superimposed upon the P wave. As a result of short recordings, the intervals

between the ectopic beats could be measured only twice and were found to be 1.1 sec. (2×0.55 sec.) and 3.7 sec. (7×0.53 sec.). The basic frequency for the parasystole would thus be about 110 corresponding to an interval of 0.55 sec.. However, the parasystole did not appear at a frequency such as would correspond to this interval. At the same time the sinus frequency varied between 100 and 120. This would implay the presence of an "exit block" or a varying stimulus formation. At the follow-up examination 2 years later long recordings were made at various frequencies, but no extra beats appeared. They would be expected to reappear if the ectopic pacemaker had still been present with unaltered frequency. This case of transitory and probable high frequency parasystole during scarlet fever has been classified as a myocarditis. There were no clinical symptoms.

Case 2 (record no. 4845/48) was an 8 year old boy with scarlet fever in the desquamation stage. Repeated electrocardiographic examinations over a period of 18 months consistently revealed the same disturbance of rhythm with parasystole. The coupling varied between 0.38 and 1.10 sec., and the intervals at rest between the extra beats were multiples of 1.03—1.12 sec., corresponding to a frequency of 53—58 a minute (see table 4). This small variation in the frequency might reasonably be expected. In the sixth record, taken after work, however, the intervals differ and on this occasion were multiples of 0.98—0.88 sec., corresponding to a frequency of 61—68 per minute. It is probable that the exertion led to the stimulation even of the ectopic center and caused this rise in frequency. No heart symptoms whatsoever were observed. It was not felt that these constant relatively low-frequency parasystole could be ascribed to a scarlet fever myocarditis.

In case no. 3 (record no. 487/48) the situation is similar. This was the case of a 10 year old boy, whose electrocardiograms, taken daily or on alternate days during his confinement in the hospital, showed infrequent ectopic systoles from the same ventricular focus with a coupling varying between 0.36—0.76 sec.. He was free of symptoms. At the follow-up examination 2 years later these infrequent extra beats were seen with couplings 0.60, 0.48, 0.48, and 0.62 sec.. The intervals between the extra beats were 5.2, 10,1, and 5.0 sec.. The frequency of the independent center must thus be at least 12 per minute but of course it could be a multiple of this in which case exit block was occurring. The low frequency is fully compatible with the appearance of the extra beats in the recordings through simple interference with the sinus rhythm. It was not felt that this constant parasystole could be ascribed to a scarlatina myocarditis either.

In two patients, only two pairs of intervals between ectopic beats were measurable, but since these were found to have a common divisor and the coupling varied considerably, it seemed a question of parasystole here too.

One case, a 4 year old girl (record no. 4593/47) showed during the illness ventricular ectopic beats with coupling varying from 0.46 to 0.72 sec.. Only

TABLE 4

Parasystole Case 2

The coupling and intervals between the ectopic beats with the largest common divisor indicated.

(The cross lines separate the values from recordings taken on different occasions.)

Curve No.	Recording conditions Notes	Coupling	Interval between ectopic beats	Divisor
I.		0.50 sec.	2.10 sec.	2×1.05 sec.
		0.50 »	2.10 »	2×1.05 »
		0.50 »	2.10 »	2×1.05 »
		0.46 »	2.16 »	2×1.08 »
		0.44 »		
II.	I—III Resting ecg. (pulse rate 75—85)	0.70 sec.	2.16 sec.	2×1.08 sec.
		0.50 »	2.10 »	2×1.05 »
		0.45 »	2.25 »	2×1.12 »
		0.50 »	5.65 »	5×1.12 »
		0.45 »	3.42 »	3×1.12 »
		0.68 »	3.10 »	3×1.04 »
		0.48 »		
		0.65 »		
III.		0.50 sec.	3.15 sec.	3×1.05 sec.
		0.70 »		
IV.	During hyperventilation (pulse rate approx. 90)	0.48 sec.	4.10 sec.	4×1.03 sec.
		0.78 »	5.30 »	5×1.06 »
		0.72 »		
V.	With pressure on the eyeballs (pulse rate approx. 75)	1.10 sec.	3.15 sec.	3×1.05 sec.
		0.66 »		
VI.	After work (pulse rate 105)	0.56 sec.	0.98 sec.	1×0.98 sec.
		0.38 »	1.90 »	2×0.95 »
		0.48 »	1.75 »	2×0.88 »
		0.44 »	1.84 »	2×0.92 »
		0.48 »	1.78 »	2×0.89 »
		0.45 »	1.76 »	2×0.88 »
		0.42 »	1.84 »	2×0.92 »
		0.48 »		

twice because of the short electrocardiographic strip could the interval between the ectopic beats be measured. On both occasions it was 1.2 sec., indicating a parasystole. Amyl nitrite during a follow-up examination 4 years later evoked two ventricular complexes, somewhat broader and higher than the normal. The P-Q time was either insignificantly shortened or unchanged. Mixed systoles would seem to have been present, the S A node and an ectopic focus, probably the earlier noted parasystolic one, discharging simultaneously. The parasystole seems to have been therefore a permanent and possibly constitutional finding. The patient has remained free from symptoms.

Case 5, an 8 year old boy (record no. 2313/49) had ectopic ventricular systoles in three of his electrocardiograms. The coupling was about 0.44 sec. on many occasions, although it could at times be found as high as 0.60 sec. The intervals were 3.04 sec. (2×1.52 sec.) and 4.50 sec. (3×1.50 sec.). It is therefore possible that this case also had parasystole. The patient has been symptom-free. On follow-up examination only normotopic systoles were observed. The somewhat doubtful parasystole was labelled as not definitely pathological. Moreover, in this patient a permanent but minor degree of intraventricular conduction defect was present.

In conclusion it may be said that only 1 out of 5 cases of parasystole was definitely ascribable to the scarlatina, one was a doubful myocarditis while the other 3 may have had constitutional basis.

Escaped Beats

Definition

The chief characteristic of the escaped beats is that they appear during unusually long intervals between sinus beats, a typically passive heterotopi. They therefore function as a physiological "substitute" stepping in when the sinus impulses are blocked. They deserve the name "Ersatzsystolen" (Wenckebach and Winterberg) and resemble the passive coronary sinus and A-V nodal rhythms. As a rule they have their origin in a portion of the heart with a naturally active automatic character — the A-V node. They reveal their kinship with the A-V nodal rhythms also by being able (alternating with single contractions) to assume the giving of the impulse for short periods and thus interfere with the sinus rhythm. They form a natural transition to the passive ectopic rhythms. Patients in whom single beat of the escaped type alternate with periods of ectopic rhythm are described under the heading of the latter. This applies to many cases of coronary sinus rhythm. When the ectopic pacemaker is in the middle or lower part of the A-V node and partial interference occurs, the character of escaped beat is so conspicuous as to warrant the inclusion of such cases also in this chapter. (No. 15—16 among A-V nodal rhythms.)

Appearance and Diagnosis

In addition to making their appearance in pathological states such as sinus arrest and A-V block they may also appear in the long but entirely normal systolic intervals of a sinus arrhythmia. They are then more probably an expression of marked "preparedness" than of pathological activity. It would often seem that their readiness to occur is too extreme because they partly interfere with the S-A rhythm. A normal P wave is then superimposed upon the extra beat thus indicating that a normal sinus beat would have taken place only a few hundreds of a second later.

While A-V nodal escapes thus seem to be physiologically normal phenomena one must be more suspicious concerning escaped beats of ventricular origin. In the latter a ventricular focus has outdistanced the A-V node with its normally higher autonomous function. The possibility of pathologically increased activity in this focus must be considered. In contrast to parasystole it would seem to lack "protective block", and it finds an opportunity to manifest itself only during long systolic intervals. When its automatic character is activated it may send out regular impulses until it is suppressed by the normal sinus stimulation, when the sinus frequency again increases.

The differential diagnosis of escaped beats should rarely be difficult. Parasystole by chance appearing late in diastole can be differentiated by analyzing the intervals in long recordings. Then it should only be a question with regard to premature beats appearing late in diastole, which, however, seems to be a rather uncommon phenomenon. It would probably be most accurate to regard extra beats during prolonged systolic intervals as escaped beats.

As might be expected, escaped beats have been described in healthy subjects as well as in those with infectious diseases (Filberbaum, Sokolow). In the latter case a higher incidence would be expected as a result of increased vegetative lability with accentuation of a sinus arrhythmia. However, a myocarditis with disturbed sino-auricular conduction and abnormal foci could also cause them, and they may be evidence of a pathological process in certain cases.

The Author's Cases

a) A-V Nodal Escaped Beats

Escaped beats of A-V nodal origin have been observed in 9 patients, all during the first 3 weeks of the illness, and in the majority of instances superimposed on the normal P wave. Sinus arrhythmia with long systolic intervals was present in every case. Sinus arrest has not been suspected, and there have been no other alterations in the electrocardiogram. Eight patients were seen in follow-up examinations at which time electrocardiogram was taken during deep breathing and pressure on the eyeballs in most cases as well as during rest. In spite of this it was possible to reproduce the escaped beats in only

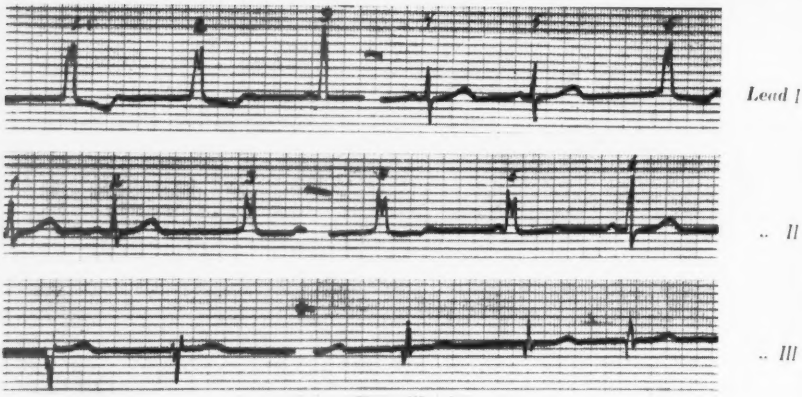


Fig. 13. *Ventricular escaped beats with short periods of ventricular rhythm. Partial interference with the sinus rhythm and appearance of mixed systoles.* (Record no. 3975/48.)

Ectopic systoles are seen in beats 1, 2 and 6, lead I; in beats 3, 4 and 5, lead II; and in beats 1 and 2, lead III. The 3rd ventricular complex in lead I is a mixed systole. The normal sinus P waves can be identified beside the ectopic ventricular complexes or merging in these.

3 of the patients. Two of these, number 15 and 16 in the A-V nodal rhythm cases, have been mentioned before (fig. 8). In the other 5 cases no escaped beats were reproduced although the systolic interval in 4 of them was just as long as the time the ectopic beats were observed. It would therefore seem that there was some relationship with the illness. It is difficult to determine, however, whether the increased irritability was due to autonomic lability or a myocarditis, and the alteration has been classified as questionable. Some time after the illness, one of the patients (record no. 4683/48) complained of breathlessness. This case is therefore recorded as a probable myocarditis with equivocal electrocardiographic changes and heart symptoms. The other patients were symptomfree.

b) Ventricular Escaped Beats

Ventricular escaped beats have been observed in 2 patients. The first of these was a 4 year old boy (record no. 3975/48) who displayed escaped beats originating from a right ventricular focus in his first electrocardiogram recorded 5 days after the onset of the illness. There was a marked sinus arrhythmia, and when the frequency fell below 75 escaped beats appeared, originating in a ventricular focus, which took over control of the ventricles for at most 3 beats in succession. (Fig. 13) The interval between the ectopic beats and the coupling were both 0.80 sec. on 5 occasions. It would therefore seem that the ectopic center had an independent regular frequency of 75. It

differs from a parasystole of the common type because it gives out impulses only late in diastole and therefore probably lacks a protective block.

The auricles were always activated from the sino-auricular node, and in most cases the P waves could be located next to the ectopic ventricular complexes. There was thus a dissociation between auricle and ventricle which was the result of the partial interference between the sinus rhythm and the ectopic rhythm and was due to the fact that the two frequencies were nearly the same. The ventricular focus never had an opportunity to activate the auricles since the sinus impulse always arrived first. This phenomenon, partial interference with secondary dissociation, is distinct from dissociation with interference.

On one occasion (the third ventricular complex, lead I, fig. 13) the sinus impulse and the impulse from the right ventricle reach the ventricles simultaneously, and a mixed systole results. The ventricular stimulus starts at the right ventricle and activates it and possibly certain portions of the left ventricle; while the sinus impulse, travelling via the usual Bundle of Hiss route, activates the left ventricle and possibly proximal portions af the right ventricle. The recording illustrates beautifully the narrow high complex corresponding to the first portion of the ectopic ventricular complex plus the R wave of the normal complex. The second R wave of the ectopic beat and S_1 of the normal complex have disappeared. Since the activation of the ventricles took place more rapidly as a result of simultaneous stimulation from two foci the entire QRS complex has been slightly shortened.

A number of electrocardiograms have been taken on this patient, the last 18 months after the illness; but the rhythm disturbance never returned. No direct cardiac symptoms have appeared either, but the patient was quite nervous immediately following the illness and experienced enuresis and night terrors. The isolated appearance of escaped beats from a relatively high frequency ventricular focus has been interpreted as an irritation — a myocarditis resulting from scarlet fever.

The second case is analogous and was a 9 year old boy (record no. 3055/47) who displayed heterotopic systoles during the first week of illness. They appeared late in diastole during the prolonged intervals of a sinus arrhythmia. Two consecutive ectopic contractions were observed, and the coupling with the preceding normal beat as well as the interval between the extra beats was constant — 0.98 sec. Normal P waves precede or are superimposed upon the ventricular complexes thus indicating a partial interference. Once a mixed systole appeared. The ectopic complexes are regarded as grouped escaped beats from a ventricular focus. The patient was examined upon 5 subsequent occasions, but the rhythm disturbance was not observed again. It has been therefore classified as a myocarditis.

Summary

In a total of 11 cases of escaped beats, 9 were nodal and 2 ventricular in origin. The latter have been regarded as myocarditic in origin while some of the nodal ones were possibly physiologically normal. It seems, however, likely that some of the nodal ones were caused by the infection, but it is difficult to definitely be established whether they were due to the resultant increased autonomic lability or myocarditis. In one case cardiac complaints suggested the presence of a myocarditis.

Paroxysmal Tachycardia

Paroxysmal tachycardia was observed only once and was not entirely typical in this case. A 7 year old boy (record no. 2235/49) displayed a ventricular tachycardia with a frequency between 120 and 140 (fig. 14 b—e) alternating with an entirely normal electrocardiogram (fig. 14 a).

Judging from the appearance of the T wave in figure 14 b, lead I, it would seem possible that the auricles were sometimes activated by retrograde stimulation from the ventricular pacemaker. The double-peaked T_1 would then be caused by a negative P_1. The interval between the Q wave and the following retrograde P wave, the Q-P interval, is 0.20 sec., while the P-Q time in figure 14 a is 0.14 sec.. This would correspond to a delay in the retrograde conduction of 0.06 sec. which might reasonably be expected. In other instances, however, (fig. 14 c) T_1 is obviously positive in the ectopic systoles and the P waves are not visible.

Figure 14 e illustrates the transition between sinus and ventricular rhythm and shows the relationship between the P and T waves. The curve was recorded shortly after figure 14 c during repeated pressure on the eye balls. The patient very likely became nervous as a result of this, and the lowering of the S-T segment and the T wave seen in lead I, figure 14 e, as compared with figure 14 c is probably related to an increased sympathetic tone. As a result the sinus frequency increased while that of the ventricular pacemaker remained unchanged, and the S-A node temporarily superseded the ectopic focus in the giving of the impulse in complexes 6 through 8. During the transitions the P wave is visible superimposed upon the ventricular complexe in beats 4 and 10. In beats 5 and 9 the impulses from the sino-auricular node and the ventricular pacemaker reach the ventricles simultaneously, and mixed systoles appear.

In complexes 1 to 3 and 11 to 13, the P waves cannot be seen. It is probable that they were not produced by retrograde stimulation on this occasion since they would then have been apparent in the T wave. In figure 14 e, however, the terminal complex has the same appearance in beats 1 through 3 and 11 through 13, in which the P waves are invisible, as in complexes 4 and 10 in which the P waves immediately precede the ectopic beats. There are no signs

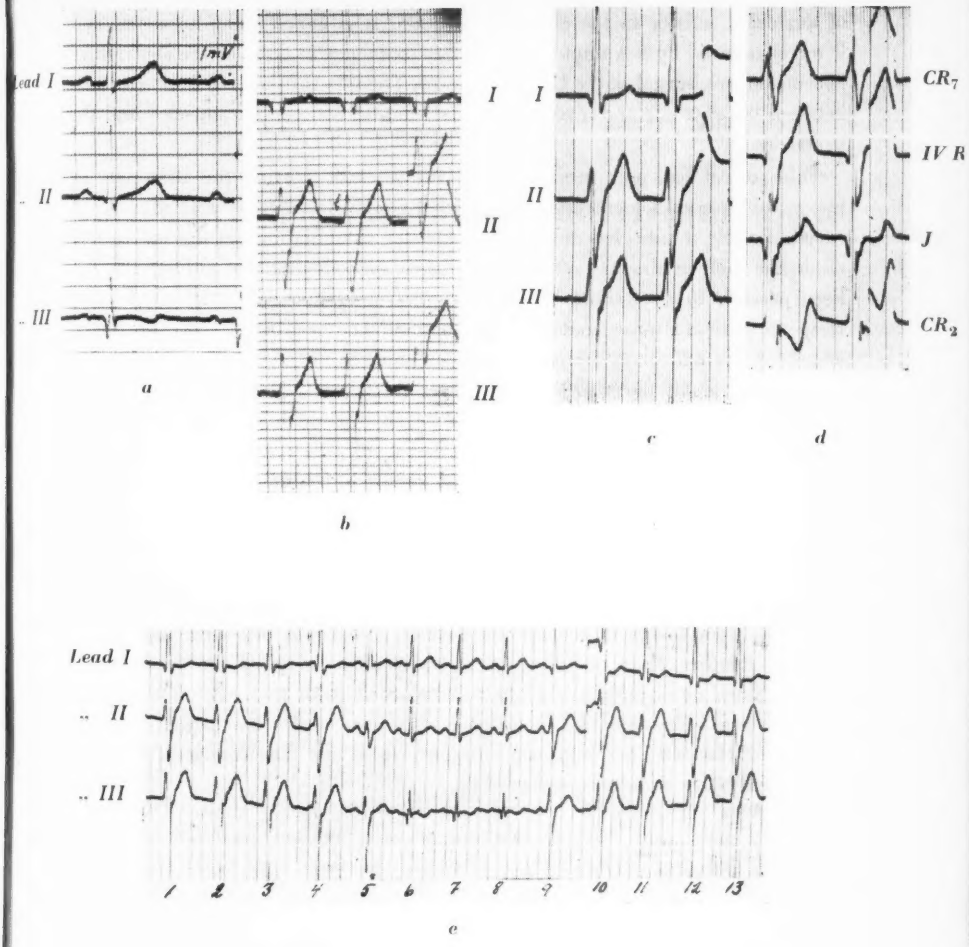


Fig. 14. *Paroxysmal ventricular tachycardia.* (Record no. 2234/49.)

a. Limb leads showing sinus rhythm with normal QRS complexes.

b. Limb leads showing ventricular tachycardia with probably retrograde conduction of the impulse to the auricles. The double apex of T_1 would seem to be due to a negative P_1.

c. Limb leads showing ventricular tachycardia without visible P waves.

d. Chest leads, CR_7, IVR, J, and CR_2, recorded at the same time as c.

e. Limb leads showing transition from ventricular to sinus rhythm. In complexes 6, 7 and 8 there is a sinus rhythm; in nos. 1 to 4 and 10 to 13 the ventricles are activated by the ventricular pacemaker; and in beats 5 and 9 there are mixed systoles. The auricular waves are not visible in beats 1 to 3 and 11 to 13. In nos. 4, 5, 9, and 10 they can be seen beside the QRS complexes.

of auricular activity in the electrocardiogram. It is conceivable, however, that P waves induced by S-A impulses of similar frequency to those of the ventricular pacemaker are buried in the QRS complexes. There is then a partial interference between the rhythms and a secondary dissociation between the auricles and the ventricles.

This patient has now been observed for 2 years. During the first part of his hospital confinement the tachycardia could usually be reproduced by exertion while it subsided during rest. At later examinations in the out-patient clinic the tachycardia has often been present even during rest although it has been possible to re-establish the sinus rhythm experimentally (fig. e). During sleep the patient's pulse (noted by the mother) has been about 70. The boy is symptom-free and plays actively like other children.

This ventricular tachycardia has a lower frequency (130) than the ordinary paroxysmal tachycardias. It also differs in that the frequency may actually be overtaken by the sinus rhythm. It is perhaps more correct therefore to call it an ectopic pacemaker related to the parasystoles. During rest and at lower sinus frequencies it seems to be temporarily latent or exit blocked.

In clinical type, with appearance during exertion, this case corresponds to the cases of paroxysmal ventricular tachycardia in patients with otherwise normal hearts published by Wilson et al. in 1932. This disturbance of rhythm, which is often a sign of serious myocardial damage, is also described in the case of another child with an otherwise normal heart (Levander-Lindgren 1949). Since no electrocardiogram was recorded prior to scarlet fever in this case the possibility that the alteration was present earlier and perhaps of constitutional character has not been definitely ruled out. The change is therefore not classified as a definite myocarditis.

CHAPTER 9

Auriculoventricular Conduction Disturbances

Bases of Interpretation

As the knowledge concerning electrocardiography has been broadened the limits of normal variation have been generally extended. This is particularly true regarding atrioventricular conduction time. As pointed out previously (page 24) the range of variation for humans between the so-called normal limits, must be distinguished from the range of variation for a single normal individual. The latter is considerably narrower and is the most important consideration in a study of this type since a diagnosis of myocarditis can be made only on the basis of changes in a series of electrocardiograms from the same individual rather than on the findings in a single recording.

The Normal Limits

It is a difficult project to establish normal limits, especially the important upper limit, for the conduction time. It should be based on large statistically treated series of normal subjects.

Lepeschkin (1942) has assembled 1500 studies on children. He has established the following normal values which will be used as a basis for interpretation. A lower limit, average value, and upper limit (in seconds) are given for each age group. The average value is italicized.

Age in years	1—2	3—5	6—9	10—14
Lower limit	0.08	0.10	0.11	0.11
Average	*0.11*	*0.12*	*0.13*	*0.14*
Upper limit	0.16	0.17	0.18	0.20

A number of authors have reported values in general agreement with the above (Mannheimer, Nadrai, Ohr, Seham, Savilahti). The upper limit is rather high, however, but is probably justifiable as shown by Mannheimer's statistically treated material in which the upper limit for older children corresponding to 3 σ is 0.20 sec.

For adults the normal range reported by Stewart and Manning for healthy men, 0.09 to 0.24 sec., statistically corresponding to $\pm$ 3 σ, has been used. For

women the normal values obtained by Grewin in his survey of the literature have been used — 0.10 to 0.22 sec. These 3 σ-limits do indeed offer quite a wide range, but as Stewart and Manning have remarked it should be remembered that 0.3 per cent of normal subjects will fall outside of these limits.

The values given here are valid only at lower frequencies. Most investigators (Seham, Harris et al., Nadrai, Ohr et al., Graybiel et al.) seem to agree that the normal value is approximately 0.02 sec. lower at higher frequencies. Graybiel et al., in their study of 1000 normal men found 0.20 sec. to be the highest value when the frequency was 80 or more. In contrast Benedetti and Savilahti found no definite difference in the P-Q time for various frequency groups. Schlamowitz has actually found a correlation between the duration of systole and the P-Q time in his analysis of the P-Q interval before and after work. This has not been statistically established, however. The correlation was greatest during rest, and it is conceivable that the rapid and extensive alterations in hemodynamics and vegetative tone following work affect the frequency and the P-Q time in unlike degree and thus disturb the correlation. In any case it would seem reasonable to consider the frequency when evaluating P-Q intervals at the upper limit of normal. Such has been the procedure in this study.

The Significance of the Variation

A definitely established prolonged conduction time justifies a diagnosis of active myocardial disease only when a series of electrocardiograms has shown that it developed from or returned to a normal value. The variation, which must exceed the physiologically normal individual range of variation, is important in the diagnosis of myocarditis. An objection to this criterion is that persistent changes which develop early in the illness could be overlooked. There should not be too great risk of this in scarlet fever. With few exceptions all cases of auriculoventricular block which were observed to develop during the illness in this study disappeared. There should probably be the same likelihood of regression in the early-developing forms of A-V block which may be the source of diagnostic difficulty. Of course one must count upon that some of the constant A-V blocks which were observed during the scarlet fever and regarded as equivocal changes are caused by persistent myocardial damage. Such myocarditical cases can hardly be diagnosed from electrocardiogram only. The possibility of a recurrence of the myocarditis should also be considered.

In the case of conduction times which are caused by myocardial disease and are thus pathological for a particular individual but which lie within the accepted normal limits, the diagnosis of myocarditis can be established only on the basis of the changes occurring from one examination to another. These cases of myocarditis may be discovered only because the individual's normal limits are exceeded. The latter are therefore of great importance.

Normal Individual Variations

Unfortunately there are only a few studies of the normal individual range of variation. Simonsson et al. (1949) have studied and statistically treated serial electrocardiograms recorded under basal conditions on 12 young healthy men. Calculating from the individual standard deviation for the P-R times, they found that the probable individual range of variation amounted to 30 per cent of the normal variation for the species. Similar values were obtained by Lewis (1912) who, however, performed only two studies on each subject. Since these values were obtained under basal conditions it must be expected that greater variations would occur during the different parts of the day and, similarly, as a result of the changing conditions of an illness.

Variations in the P-Q time must be regarded as an expression of adaptation to various functional demands—a resultant of constitution and environment. They may therefore be of different magnitude in different individuals and would be greatest, on a purely physiological basis, in subjects performing heavy labor or active athletics which place a greater demand on the adaptability of the circulation.

While earlier studies have been made in which variations of as little as 0.02 sec. have been regarded as significant (Wickström), in this study greater variations have been demanded in accordance with later experience. At the same or higher frequencies prolongations of the conduction time of 0.04 sec. or more, and at lower frequencies prolongations of 0.05 sec. or more, have been regarded as suspicious of myocarditis. It is felt that even at different frequencies the normal range of variation seldom exceeds 0.05 sec., and Savilahti regards a variation of 0.04 sec. as being more significant than a single high value. This value corresponding to approximately 50 per cent of the variation for children should be an acceptable basis for the evaluation of the individual range of variation. In subjects who normally have a narrower range of individual variation, isolated pathological values may be overlooked; but, on the other hand, a drawing-in of the limits would certainly do more damage and would result in overdiagnosis.

While a variation of 0.04 sec. or more has been regarded as suspicious of myocarditis the diagnosis has been established only after a study of the clinical course and the serial electrocardiograms in their entirety. In cases where there was reason to suspect an unusually wide range of normal variation or an extreme normal value lying outside of the accepted limits, a follow-up examination has been made 1—4 years after the illness. At this time myocarditic changes would probably have regressed. By means of a series of rest and work electrocardiograms an effort has been made to establish the individual's physiological range of variation and reproduce the suspicious value. The functional capacity may also be established by means of the function test. Of course clinical symptoms are also considered, but a lack of these is not regarded as ruling-out a diagnosis of myocarditis. In this way it should be possible to distinguish lesions

not obviously related to the scarlet fever or constitutional extreme variations both with regard to the absolute values and the range. The latter are often reported in the literature as "functional A-V prolongations", but the name does not seem to be entirely suitable since also pathological prolongations of the A-V conduction time are often functionally variable. Unfortunately the final diagnosis is delayed because of this, and the patient must be treated as a suspected myocarditis during the interval.

P-Q Variations Within a Single Recording

In addition to the above-mentioned variations from one recording to another variations from one beat to another in a single curve may sometimes be significant. An A-V lesion may manifest itself in abnormally large variations of this type as pointed out by Wenckebach and Winterberg (1927) and Groedel and Kisch (1940). (Compare also with grade II A-V block.) When the P-P interval is constant the variation may not exceed 0.02 sec. according to Segers. He does not state how large it may be in sinus arrhythmia, but as is the case from one recording to another variations of 0.04 sec. and more are regarded as suspicious.

In different recordings at different frequencies the longer P-Q intervals are usually found at the lower frequencies which, like constitutional and experimental vagotonia, cause a prolongation of the conduction time. It is thus influenced by autonomic factors, and longer P-Q times seem to be associated with increased vagus effect. Wenckebach periods have also been described in a young man with respiratory sinus arrhythmia but an apparently normal heart (Öhnell and Andersson). In this case the longer P-Q intervals coincided regularly with the longer P-P intervals, and a ventricular beat was lost during rest with each expiration. It was felt that an increased nervous sensitivity was present. Ljung (1951) has described latent A-V block which appeared during deep breathing in cases of myocarditis.

Restitution is probably another factor of significance in fluctuations in conduction within a single recording. It will be more complete the longer the time elapsing after the preceding contraction. Thus Segers and van Heerswyngels in a case of complete sinus arrhythmia have been able to show that the longer the P-P interval the shorter the P-R time.

In the individual case the relationship between a P-Q variation and the P-P interval will depend upon which of the two factors, vagus effect or restitution, has the greatest effect. It would seem questionable whether any great variation in the vagus effect on conduction ordinarily occurs during a respiratory arrhythmia, since the vagus stimulation is greatest at the sino-auricular node and diminishes distally. Observations in this material indicate that restitution ordinarily has the greater influence. In a number of cases it has been found that the longer P-Q times have occurred with the shorter P-P intervals during sinus arrhythmia. The reversed relationship was never observed.

As might be expected a defectively functioning conduction would be more

dependent on restitution than the normal and the P-Q fluctuations greater than usual in such a case. In 2 children with prolonged auriculoventricular conduction in this study fluctuations of from 0.12 to 0.19 sec. and 0.13 to 0.21 sec. have been observed in recordings made during rest. Such cases display a certain similarity to second degree block of the Wenckebach type, but the variation, depending as it does upon the systolic interval, is possibly an expression of extreme but physiologically normal lability in certain cases. Even if the P-Q variations seem to be abnormal it is possible to determine whether the cause was a myocarditis only after consideration of the course.

A 6 year old girl (record no. 3619/48) showed a marked arrhythmia in a rest recording with the P-P interval varying from 0.60 to 1.10 sec. The corresponding P-Q times were 0.17 and 0.13 sec. respectively. In previous electrocardiograms the fluctuations of the P-Q time did not exceed 0.02 sec., and the maximum values were 0.14 to 0.16 sec. At an examination one year later with rest and work electrocardiograms the P-Q time varied only between 0.12 and 0.14 sec. The fluctuation of 0.13 to 0.17 would therefore seem to be suspicious, but because of the fact that the arrhythmia was extreme and the maximum values did not exceed normal limits it was not felt that a diagnosis of myocarditis was justified.

Technique of Measurements

The measurements have been made on lead II, and the variation or the longest interval has been reported. The comparison between different electrocardiograms has been made, as a rule, on the basis of the greatest values in the different curves. In isolated cases with large variations in the same recording the comparison has been made between the extreme values within the one recording. Variations resulting from changes in the P wave have, of course, been omitted. An illustration of the latter is provided by a 9 year old girl (record no. 800/48) who had a P-Q variation from 0.11 to 0.17 sec. (fig. 15). In this case P_2 variations occurred simultaneously with the fluctuation in the conduction time, indicating a wandering pacemaker.

Classification

In addition to the usual classification of auriculoventricular block into grades I, II, and III it seemed desirable, for practical reasons and for purpose of summarizing, to subdivide grade I block according to the previously established interpretation into two groups, A and B. Group A includes those cases in which the absolute value and the range of variation both exceed the normal limits. In group B are placed those cases where only the range of variation and not the absolute value exceed the normal limits.

For comparison and to illustrate the procedure for interpretation, the changes in the P-Q interval which were not regarded as indicative of an active process have also been recorded. These were also divided into two groups, group C including those with P-Q times above the normal limits and group D those with a range of variation exceeding the normal limits.

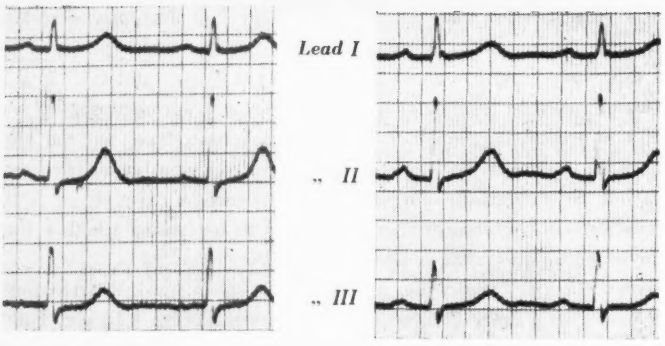


Fig. 15. *Variations in the P-Q time associated with wandering of the pacemaker.* (Record no. 800/48.)

a. Perisinus rhythm with a P-Q time of 0.11 to 0.12 seconds one minute after work.
b. Sinus rhythm with a P-Q time of 0.16 to 0.17 seconds 3 minutes after work.

Grade III A-V block		
Grade II „ „		
	Group A. Both the highest value and the range of variation exceeding the normal limits.	*Myocarditic cases with changes related to the scarlet fever.*
Grade I „ „	*Group B.* Highest value within but range of variation exceeding the normal limits.	
	Group C. Highest value above the normal limits.	*Equivocal cases with changes not obviously related to the scarlet fever.*
	Group D. Highest value within but range of variation exceeding the normal limits.	

Even when it is possible to give the limits in actual figures, as for instance with the P-Q time, there are always questionable cases because of the variety of factors which influence the electrocardiogram. The difference in individual variation from one subject to another is not the least of these factors. Such cases will be discussed further in the various groups. If at the same time there were subjective complaints referable to the heart a myocarditis was probably present. These patients have been presented under a special heading—Probable myocarditis with symptoms—as in other cases with changes in the electrocardiogram in which the symptoms contributed to the diagnosis. All cases with A-V conduction disturbances, ascribed to scarlatina myocarditis, are listed in table 5.

TABLE 5

CASES OF MYOCARDITIS WITH A-V BLOCK

Classification by groups according to the author's description on pages 87 and 88

Type of block	Number of cases	Time of onset				Duration[1]							
		1st wk.	2nd—3rd wks.	4th—5th wks.	Later	1×[2]	1 wk.	2—4 wks.	5—8 wks.	3—5 mos.	6 mos. —1 yr.	1 yr.	Unknown
Grade III	1			1		1							
» II	2	1			1	1					1		
» I Group A	27	6	7	10	4	10	6	3	3	1	1	2	1
» B	4		1	2	1	3	1						
Group D (with symptoms)	1	1				1							
Total	35	8	8	13	6	16	7	3	3	1	2	2	1

1 As judged by the rest electrocardiogram. Two patients had abnormal work reactions even later, and there were 3 cases with a recurrence of the A-V block at a later time after recovery from scarlet fever.

2 Observed only on one occasion.

CASES IN THE PRESENT STUDY

I. Conduction Disturbances Ascribed to Scarlet Fever Myocarditis

Grade III A-V block has been observed in this study only one time, and only isolated cases have been reported in the literature on scarlatina (Toomey and Seecof, Paul and Rhomberg). Our case is interesting since it illustrates how transitory even a serious alteration may be. This was a 29 year old woman (record no. 147/48) with a toxic scarlatina. She belonged to the control group and did not receive penicillin, but since she was in a toxic state at the time of admission she received convalescent serum. At the time of onset she had a transitory synovitis. The first evidence of myocarditis appeared in the third week when T_1 became temporarily isoelectric. During the fifth week she complained one day of pain of the angina pectoris type. She was rather ill, the pulse was slow but regular, but the radial pulse beats were of varying strength. The intensity of the first heart sound was also variable on auscultation. The systolic blood pressure had fallen to 80 mm.Hg. The electrocardiogram was recorded immediately and showed a total A-V block. The variation in the strength of the first sound, which is partly dependent upon the P-R interval, may be explained by the dissociation between the auricle and the ventricle with the resulting variation in the time relationship between P and QRS (Wolfert and Margolies 1930, Dock 1933, Beard and Decherd 1947, Levine 1949). By the next day the conduction time was again normal and remained so. A follow-up examination was made after two years, at which time the patient stated that immediately following the illness she had noticed a marked general fatigue but that subsequently she had felt about as usual. She reported no shortness of breath or precordial pain. Six months previously she had experienced a normal pregnancy and was also the housewife in a rural home with heavy work. The blood pressure was 110/70, and the heart tones were clear and the rhythm regular. The electrocardiogram was normal both during the rest and after exertion, and she was able to manage 600 Kg.M./min. in the function test — normal working capacity. The x-ray of the heart was not remarkable. In this case there was thus a transitory total A-V block with complete recovery.

Grade II A-V block was observed in 2 patients. In the first case there was a prolonged change and in the second a temporary one.

The first case was that of an 18 year old woman (record no. 2496/47, series C_1). She had a high fever at the beginning with a temporary synovitis on the fourth day of illness and at the same time precordial pain. The first electrocardiogram was recorded on the seventh day of the illness and showed grade II A-V block with Wenckebach's periodicity. This block remained unchanged during the 6 months of the patient's hospital stay. During the fifth week of illness prolonged fever developed with joint complaints and a high sedimentation rate. This would seem to be most readily explained as a complicating

rheumatic fever. At this time penicillin therapy was instituted. A check-up 3 months after discharge (9 months after onset) showed that the P-Q time was normal and constant at 0.14 seconds. The patient was troubled by palpitations and dyspnea, but despite this she was able to perform relatively strenuous work as a ward helper during the following year. She subsequently became entirely free of symptoms and was able to participate in sports just as before the illness. Three years later she went through a pregnancy and delivery without any trouble from the heart. Six months later, however, she began to have dyspnea and angina pectoris on exertion although no known infection or arthritis was observed. At this time the electrocardiogram displayed sinus tachycardia but was otherwise unremarkable both at rest and at work. Her symptoms continued and she had difficulty in performing heavy housework, and for this reason she was examined again one year later. At this time she was found to have sinus tachycardia and grade II A-V block with Wenckebach's periods alternating with normal conduction. These phenomena were also observed immediately following work. The sedimentation rate was normal and there were no clinical signs of myocarditis. The x-ray of the heart was normal, and the basal metabolic rate was plus 12 per cent. In this instance the course of illness would seem to indicate a myocarditis, and probably also a rheumatic infection, during the scarlet fever infection. After two years of freedom from symptoms the heart changes would seem to have recurred for unknown reasons.

The second case (record no. 841/49) was that of an 11 year old boy. Twenty-five days after the onset of illness he was admitted in the desquamation stage. At this time he had a fever of 39°C. for 3 days, and hemolytic streptococci were cultured. For this reason penicillin therapy was instituted. The electrocardiogram was normal. At a follow-up examination in the seventh week he was again found to have hemolytic streptococci, rhinitis, and sinusitis and was readmitted because of the risk of contagion. The first electrocardiogram during this admission was misplaced, but two subsequent recordings were normal. In this case the chief clinical interest was in the sinusitis, and no evidence of heart involvement was observed. Some time later the misplaced electrocardiogram was located and found to show grade II A-V block with Wenckebach periods. This serious alteration had thus produced no objective clinical findings and had rapidly disappeared. At a follow-up examination one year later, however, it was reported that during the first 6 months after the illness the patient had been rather weak and easily tired but without actual cardiac difficulties. The function test and the rest and work electrocardiograms were not unusual.

Grade I block, group A

In this group the absolute P-Q value exceeds the normal limits, and the range of variation is greater than 0.04 seconds or equal to 0.04 seconds with the greater value at unchanged or higher frequency. Even among the cases which meet both of these criteria it should be possible to find individual ones which

are not due to a myocarditis, especially amongst those with the smaller ranges of variation, i.e. 0.04 and 0.05 seconds. In order to justify the diagnosis of myocarditis it has been agreed that the abnormal values should differ noticeably from the others in the series. If this was not the case the alteration has been included in group C. In addition the abnormal P-Q time and variation have been required to have developed during the scarlet fever and/or to have subsequently regressed. Otherwise the cases have also been included in group C. The period of observation in most cases has extended for one year.

In accordance with these criteria 27 patients have been assigned to group A. and the range of variation is as follows:

0.04 seconds in 2 cases
0.05 „ „ 3 „
0.06 „ „ 5 „
0.07 „ or more in 17 cases.

The range of variation is thus considerable, at least 0.07 seconds in the majority of cases. Further particulars are given below regarding the two patients with variations of only 0.04 seconds and one of those with 0.05 seconds.

The first of these was a 4 year old girl (record no. 4119/47) who in the fifth week of illness developed a prolongation of from 0.14 to 0.18 seconds which subsequently diminished to 0.15 seconds. Eleven months later a work electrocardiogram was recorded, and at this time the P-Q time of 0.15 seconds in rest increased to 0.18 seconds in spite of an increase in frequency. This pathological reaction would seem to indicate that a latent damage was still present and supports the diagnosis of myocarditis. Her values were as follows:

Frequency:	85	100	100	95	100	100	After	115
P-Q interval in seconds:	0.14	0.15	0.14	0.18	0.15	0.15	work:	0.18

The other case was also a 4 year old girl (record no. 4715/47) with a similar alteration. In this case the work electrocardiogram two years later was entirely normal:

Frequency:	85	80	100	85	85	100	After	80	After	
P-Q interval in seconds:	0.16	0.14	*0.18*	0.16	0.14	0.14	2 yrs:	0.16	work:	0.13

The case of an 8 year old boy (record no. 3868/47) illustrates the way in which a minor isolated divergence may be significant. Eight electrocardiograms were recorded in this patient, and in seven of them the P-Q time was 0.14 or 0.15 seconds. In the fourth recording, however, made in the fifth week, the P-Q time had increased to 0.19 seconds at the same frequency. Two years later the P-Q time was 0.16 seconds and unaltered by the work test.

The Course

Normalization of the P-Q time occurred in all but 3 of the patients. In addition there were 2 patients whose rest electrocardiograms were normal at the follow-up examinations but whose work reactions were abnormal with prolongation of the conduction time immediately following work. One of these has been described previously. The other was a 13 year old boy (record no. 3309/47) who was found to have a P-Q time of 0.16 seconds with a frequency of

65 during rest at the follow-up examination one year later. Immediately after work the interval increased to 0.24 seconds at a frequency of 110. Two years later a prolonged conduction time was present even at rest—0.28 seconds. This probably is a case of permanent conduction damage which was latent during rest at the time of the first follow-up examination.

In two patients normalization took place during the usual period of observation, but a later follow-up examination revealed a prolonged P-Q time again. One of these was a 5 year old boy who happened to return for examination during a new attack of myocarditis with a recurrence of A-V block. He later showed a normal P-Q time again. The other case was an 8 year old girl (record no. 4162/47) who showed a prolongation of from 0.14 to 0.22 seconds in the third week of illness. The alterations occurred in recordings showing a marked sinus arrhythmia. A variation of from 0.16 to 0.22 seconds was present in one recording and of from 0.14 to 0.20 seconds in the other and the longer P-Q intervals were associated with the shorter P-P intervals. The serial electrocardiograms showed that a normalization occurred after 3 weeks. Despite sinus arrhythmia no greater changes were found, and the P-Q time was 0.16 to 0.19 seconds. At a follow-up examination two years later, however, the conduction time was again found to be abnormally long—0.18 to 0.22 seconds in rest and 0.14 to 0.17 seconds after work. It might therefore be asked if this was not a case of extreme normal variation. On the other hand the girl had unquestionably experienced heart symptoms. One night she awoke because of difficulty in breathing. She tore at the collar of her night clothes and felt that she could not get air. It seems possible that there was a high grade block at this time and possibly an intermittent myocarditis. On one or two occasions she suddenly became very fatigued while doing gymnastics. This would seem to be such strong evidence of heart disease that a diagnosis of myocarditis is felt to be justified. A residual A-V prolongation is present, but adaptation seems to be satisfactory at least at the work load which was attempted.

One of the three cases of persistent A-V prolongation was a 36 year old nurse (record no. 2213/47) who was observed for only 3 months, subsequent to which she moved away from the city. This patient developed a prolongation of the P-Q time of from 0.16 to 0.22 seconds at a frequency of 80, and she complained of anginal pain and palpitations. It is probable that a longer period of observation would have revealed regression in this case also. The remaining cases are two children who had abnormally long conduction times during the entire observation period. In these, however, certain circumstances suggest that the alterations developed during the scarlet fever. A 5 year old girl (record no. 3653/48) had a P-Q time varying between 0.12 and 0.19 seconds, longer at the shorter P-P intervals, in her first electrocardiogram. Subsequent to this the value was consistantly high during rest, 0.18 to 0.20 seconds, and somewhat shorter after work. One and a half years after the illness the P-Q time was still 0.22 to 0.23 seconds in rest and 0.20 after work. Two and a half years later

there was a sinus arrhythmia during rest with a frequency of 55 to 80. At this time the P-Q interval was 0.17 to 0.22 seconds. During work, at a frequency of 135, it was found to be 0.18 seconds. At this time the patient was alert and active and played as other children, but for the first year after the scarlet fever she had been somewhat dyspneic. A systolic murmur was heard on auscultation, but this did not seem to have an organic character. The x-ray of the heart was normal. In this case there was a persistently prolonged conduction time of great variability. It must be asked whether this was a constitutional anomaly or an old lesion. The great variation in the first electrocardiogram and the values as low as 0.12 seconds which never reappeared would seem to suggest that some damage to the conduction system developed at this time. On the first occasion the lesion was relative with a variable conduction disturbance which later became more constant in character. Some adaptation to exertion could be observed in the shortening of the P-Q time, but it is not impossible that a high grade block might develop with more strenuous exertion (Reindell and Kleipzig). The patient is allowed to play as other children but should have a yearly check-up.

The second case was that of a 6 year old boy (record no. 1776/49) who showed a conduction time of 0.23 seconds at a frequency of 95 as early as the fourth day of illness. The serial electrocardiograms showed the following:

	In rest		After work	
Time after onset:	Frequency	P-Q time (sec.)	Frequency	P-Q time (sec.)
4 days	95	0.23		
11 „	55—100	0.13—0.21		
18 „	90	0.18—0.19		
5 weeks	85	0.20—0.22	120	0.16
6 „	90	0.18—0.24	100	0.18
7 „	95	0.19—0.20	105	0.15—0.16
6 mos.	95	0.22	110	0.17
1 yr.	95	0.24—0.25	110	0.21
2 yrs.	75	0.28	During work:	0.16
			After work:	0.16—0.20

In this case there was an abnormally long, as well as an abnormally variable, P-Q time. It diminished after exertion but did not always reach a normal value. An A-V prolongation was present even in the first electrocardiogram. The shortest P-Q time was found in the patient's second electrocardiogram, at which time it varied from 0.13 to 0.21 seconds (figure 16). A value this low was not observed again, however, and it would thus seem that some auriculoventricular damage developed during the scarlet fever. The marked variation in rest seen in the patient's second electrocardiogram probably is an indication that only moderate, functionally variable A-V injury was present. Since the prolongation of the A-V time persisted, however, the diagnosis of myocarditis has been made

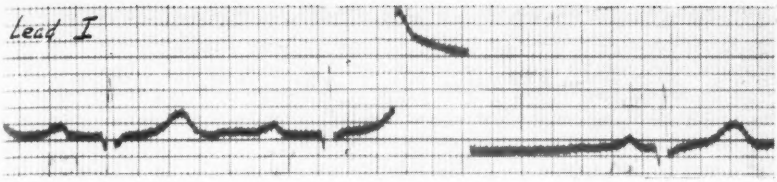


Fig. 16. *P-Q variations with sinus arrhythmia.* (Record no. 1776/49, lead I.)
The P-Q time varies between 0.13 and 0.21 seconds. It is shorter when the systolic interval is longer.

with some doubts and a reservation as to the possibility of a constitutional anomaly or an old lesion. At the time of the follow-up two years later he had just undergone an appendectomy as a result of mesenteric lymphadenitis. The further prolongation of the conduction time may have been caused by an irritation due to the lymphadenitis and possibly a resultant relapse of the myocarditis. The mother noticed that following scarlet fever the patient was fretful and impatient and lacked his former endurance. Following the operation, however, he was brighter and more cheerful than previously.

Grade I A-V block, group B

In this group are included cases in which the upper normal limit was not exceeded but in which the range of variation was larger than normal, i.e., greater than 0.04 seconds, or equal to 0.04 seconds if the higher value did not occur at a lower frequency. The alteration must differ noticeably from other values in the electrocardiographic series. If these criteria were met the variation was regarded as evidence of myocardial damage. Otherwise the variation was included in group D.

This group includes only 5 patients. One of these also displayed inverted T waves. Of these, 3 had a range of variation of 0.04 seconds; 1, one of 0.05 seconds; and 1, a range of 0.06 seconds. The following cases are offered as illustrations:

A 32 year old housewife (record no. 4084/48) showed a short P-Q time (0.12 seconds) in her first 3 electrocardiograms. At the first follow-up in the fifth week this had increased to 0.16 seconds at the same frequency of 75 beats per minute. Although the value was still relatively low it was regarded as suspicious on the basis of the variation. A work electrocardiogram was recorded as a control, and the P-Q time in this was still 0.16 seconds in rest. After work, at the increased frequency of 120, the P-Q time increased further to 0.20 seconds. On the basis of this variation and the abnormal work test reaction a diagnosis of acute myocarditis was made. In a number of subsequent recordings the conduction time was again 0.12 to 0.13 seconds and after work 0.11 to 0.12 seconds. In addition to this the woman had considerable subjective difficulty. Immediately following her illness she was short of breath even with minor exertion, and she also noticed precordial pain at the same time. The latter was also experienced at night when it sometimes awakened her. In con-

trast to her usual practice she began to sleep with her head elevated, and subsequent to this the difficulty diminished. At a follow-up examination one and a half years later she no longer slept with her head elevated, was free of precordial pain, but was still unable to do the heavier tasks in the household such as the laundry. She was able, however, to manage 600 Kg.M./min. in the function test, and both the rest and work electrocardiograms were normal.

A 2 year old girl (record no. 1505/48) developed scarlet fever on the basis of infected burns. In the electrocardiograms recorded two or three times a week during the illness her P-Q time was 0.10 to 0.12 seconds with the exception of a single recording made on the sixteenth day of illness. On this occasion the P-Q time was 0.16 seconds at the same frequency. At the follow-up examination 2 years later the P-Q time was 0.11 to 0.12 seconds in rest and 0.10 to 0.12 seconds after work. This change of 0.04 seconds would seem to be definitely aberrant and suggestive of myocarditis. This patient has not been counted in the total of myocarditis cases since the aberration was not observed in the routine electrocardiograms but was found because of more frequent electrocardiographic examinations.

The following case is also a good illustration of how a conduction time within normal limits may assume a significance as a result of the variation. This was a 3 year old boy (record no. 1017/49).

	During the illness						After 1 year	
							Rest	After work
Frequency	85	115	120	135	120	85	80	90
P-Q time in seconds	0.12	0.17	0.12	0.13	0.12	0.12	0.12—14	0.10—12

The alterations in this group are relatively small; and they have regressed completely in all cases, which, to a certain extent, has been one of the prerequisites for this classification. Noteworthy symptoms have persisted, however, in the case of one woman, and even after 2 years she did not seem to have regained her usual capacity although her function test was within normal limits. One of the children has also been reported as being more short of breath than usual after the illness, but he had gained a considerable amount of weight, and for this reason the symptom may be questioned as a sign of myocarditis.

Summary of the Myocarditic Atrioventricular Blocks

Thirty-four patients had a prolongation of the conduction time, which was interpreted as evidence of myocarditis. Of these, 13 were adults and 21 children, corresponding to 4.67 per cent and 0.82 per cent respectively. The difference, 3.85 ± 1.3 per cent, is quite definitely established.

The cases are distributed somewhat unequally so far as the different groups are concerned. Thus there were 1.2 per cent in the series treated with penicillin, 1.9 per cent in the control series, and 1.7 pr cent in those cases not included in the regular series. In the desquamating group there were only 3 cases, corresponding to 0.6 per cent. All of these had marked alterations: 1 second degree block and 2 prolongations of 0.10 seconds. The lower incidence here is probably a result of the fact that these patients were observed for a shorter period of

time with fewer electrocardiograms and that only the more marked changes were observed. It should be noted that grades II and III block have occurred only in the cases who received penicillin late in the illness or not at all.

Symptomatology

Subjective complaints have been reported by 12 of the patients. These consisted of anginal pain in 3 of the women and 3 of the girls, dyspnea in 7 patients, and palpitations in 2. General fatigue, which has been present in another 8 patients, has been regarded as too nonspecific to be counted. On the other hand, if it has been reported that a child suddenly and without reason has gone and lain down during play it has been regarded as significant. The mothers of two children with prolongation of the atrioventricular conduction time have spontaneously reported this symptom during the period immediately following the illness. Usually the complaints have persisted longer than the changes in the electrocardiogram. However, even in the presence of more marked changes, a few of the patients have been free of symptoms and able to get up and about. In 2 of them the function test during the myocarditis showed the same value as two or three months later after recovery, and the P-Q time was shortened in the work electrocardiogram although not to the normal value.

Onset and Duration

Table 5 summarizes these facts. The different dates of onset extend over a long time range, but in no case did the block occur later than the seventh week. The great majority of changes have been of short duration—one week or less. Since electrocardiograms were not recorded more than once a week as a rule, the alterations may actually have been present for somewhat longer periods of time, but they have been actually demonstrated for only 8 days. It is worth noting that even serious A-V disturbances have been transitory, and there does not seem to be any close relationship between the magnitude and the duration of the changes. There have probably been lesions of various types—toxic, as well as structural, and circulatory. All of the minor aberrations in group B have been of short duration, i.e. one week or less.

The clinical course is described under the various groups.

II. Conduction Disturbances not Obviously Related to the Scarlet Fever

As mentioned before, disturbances of the conduction time which do not seem to be indicative of an active myocardial lesion are also to be discussed here. By this means the interpretation, as well as aberrations which may be seen in seemingly normal people, are clarified. Filberbaum et al. (1946) also report that such changes may be found in people with normal hearts.

Group C. Prolonged Conduction Times

This group includes 26 patients with conduction times exceeding the normal limits. The great majority exceed the limit only slightly—17 by 0.01 seconds, 8 by 0.02 seconds and only one by 0.03 seconds. The range of variation was within normal limits $\leqq$ 0.03 seconds, in 22 patients. The conduction time in these cases thus appeared to be constant during the period of observation, which in the majority of cases was at least one year, and it was felt that there was no relationship between the phenomena and the scarlet fever.

A curious phenomenon was observed in the case of a 5 year old boy (record no. 4545/48). During the illness his P-Q time varied between 0.16 and 0.18 seconds, and at the time of the follow-up examination 3 years later the same value was found at rest. On deep breathing, however, the P-Q interval varied from 0.12 to 0.20 seconds without any particular change in frequency.

In 4 cases a wider range of variation was present during the illness, amounting to 0.04 seconds in 3 cases and 0.07 in 1. The follow-up examination was of decisive importance in interpreting these cases. Since both the high values as well as the wide ranges of variation were again equaled, or nearly equaled, it would not seem that these aberrations were a definite result of an acute scarlatina myocarditis either. As an example the following series from a 5 year old girl (record no. 4250/48) are offered:

Frequency:	85	100	90	95	80	95	After work:	100	75
P-Q time in seconds	0.14	0.15	0.12	0.19	0.18	0.16		0.11	0.11

At follow-up examination 2 years later:	In rest:	After work:
	70	110
	0.18	0.12

The values 0.18 and 0.19 seconds, at a relatively high frequency during the illness, differ, of course, from the remaining values; but in view of the finding at the time of the follow-up examination (the wide range of variation) it would seem that this may have been related to a marked constitutional variability. This and a similar case (record no. 3581/47) have been listed as "questionable myocarditis". These patients were free of symptoms.

These prolonged conduction times may be due to old lesions or to constitutional aberrations. A persistent damage from the scarlatina seems less likely. In such cases it is difficult to determine where the borderlines should be drawn between extreme variations of the normal and congenital A-V block. Several cases of congenital total A-V block are described earlier. Nothing in the history of these children has been suggestive of old lesions, and this would be rather unlikely in the case of such young subjects anyway. A prolongation which on all probability is due to hereditary constitutional factors is well illustrated by three groups of brothers and sisters in this material.

In one of these groups the 3 siblings had scarlatina 2 years in succession and were followed by electrocardiography for 3 years. The following chart shows their ages and P-Q times during this period

1) Girl	Age:	2 yrs.	3 yrs.	4 yrs.
(rec. no. 4085/48)	P-Q time:	0.16 sec.	—	0.18 sec.
2) Girl	Age:	5 yrs.	6 yrs.	7 yrs.
(rec. no. 4086/48)	P-Q time:	0.16—0.17 sec.	0.17—0.18 sec.	0.22 sec. (after work, 0.20 sec.)
3) Boy	Age:	7 yrs.	8 yrs.	9 yrs.
(rec. no. 4114/48)	P-Q time:	0.18—0.20 sec.	0.18—0.20 sec.	0.22 sec. (after work, 0.18—0.20 sec.)

Their maximum values lie from 0.01 to 0.04 seconds above the normal limits, and the increases would seem to be related more to the age than to the scarlet fever. During the increase observed between the second and the third observation year all had been in good health. It would seem that in all probability these are cases of constitutional hereditary conduction disturbances. It is unlikely that there may have been a myocarditis producing persistent damage in all 3 children. The decrease after work is rather small, and the mother feels that the children are not in very good physical condition. They are inclined to rest often during walks although not more following the scarlatina than before. A fourth sibling in the family has a better physical working capacity and a normal electrocardiogram. The mother's conduction time was 0.18 seconds. The father was not examined.

Another pair of siblings also had a prolongation of the P-Q time. The girl (record no. 4144/47), who was 10 years old, had a P-Q time of 0.18 to 0.19 seconds while suffering from a streptococcus sore throat. Two and a half years later at a follow-up examination this was 0.19 to 0.22 seconds and decreased to 0.18 to 0.20 seconds after work. During the interval she had been entirely well.

The brother, who was 8 years old, had a P-Q time of from 0.18 to 0.20 seconds during scarlatina, and this was the same after two and a half years. The prolongation of the conduction time in the girl has occurred without relationship to any illness and would seem to be an entirely physiological phenomenon due to development. It thus seems that a constitutional aberration from the normal also exists in this pair of children. The mother had a P-Q time of 0.20 seconds.

In still another family a 2 year old boy (record no. 4674/48) was found to have a slight prolongation of the conduction time to 0.17 seconds. A 6 year old sister and 10 year old brother had 0.17 and 0.18 seconds respectively—values thus lying at the upper limits of normal.

These brothers and sisters appeared for examination during the period of the study quite by chance when suffering from either scarlet fever or some other streptococcus infection. The siblings and parents of other children in this group could not be examined because of practical difficulties. If this had been possible several families with such aberrations would probably have been discovered. An early mild A-V lesion of lasting character can not be ruled out with certainty of course, but a diagnosis of acute myocarditis would not seem to be justified and there has been no heart symptoms.

Group D. Range of P-Q variation exceeding the normal limits

This group includes 12 patients, whose variations were 0.04 seconds, 3 patients with variations 0.05 seconds and 2 patients with 0.06 seconds. The diagnosis and differentiation of these cases from the ones in group B have been based on the appearance of the suspicious interval in the serial electrocardiograms and on whether the elevated value and the marked variation could be reproduced at later examinations.

The following series showing a gradual change in the P-Q time is regarded as not being significant. This was an 8 year old boy (record no. 3912/47):

Frequency:	100	75	85	100	90	100	80
P-Q time in seconds:	0.13	0.14	0.15	0.16	0.16	0.17	0.16

The follow-up examination has been important in making the diagnosis in some cases as for example in that of the following 10 year old girl (record no. 2044/49):

	During the Illness								After 1 yr. Rest	After 1 yr. After work
Frequency:	75	120	75	75	80	75	85	85	75	100
P-Q time in seconds:	0.16	0.19	0.18	0.16	0.20	0.16	0.18	0.16	0.20	0.16

In this case the values of 0.19 and 0.20 seconds would seem to be very suspicious particularly if the neighbouring values and the frequency are considered. The patient, who was described as being alert and unusually well following scarlet fever, had again after one year the long P-Q time of 0.20 seconds in rest and a variation following work of 0.04 seconds. The suspicious value during the illness may therefore have been a normal variation, and the diagnosis of myocarditis may be questioned.

The same situation existed in the case of the following 5 year old girl (record no. 247/49):

	During the Illness				After 1 yr. Rest	After 1 yr. After work
Frequency:	70	80	85	110	110	
P-Q time in seconds:	0.13	0.14	0.17	0.14	0.17	0.14

The isolated value of 0.17 seconds during the illness as well as the variation of 0.04 seconds would seem to be very suspicious. The value was reproduced at a later examination, however, and would therefore seem to be within the patient's normal range of variation.

A 5 year old girl (record no. 4370/48) displayed unusually wide variability (0.12 to 0.17 seconds) during the illness. Her series was as follows:

Frequency:	95	80	100	85	75—100
P-Q time in seconds:	0.12	0.17	0.16	0.14	0.15—0.17

She was free of symptoms following the illness, and at the follow-up examination 3 years later she displayed a wide variability from 0.12 seconds in the standing position following work to 0.20 seconds during deep respiration. It was concluded that she probably had a marked constitutional lability regarding P-Q time.

In rest	Deep breathing	Standing	Standing after work
0.14—0.18 sec.	0.16—0.20 sec.	0.14 sec.	0.12 sec.

A 19 year old youth (record no. 603/48) had a variation in the P-Q time of from 0.18 to 0.24 seconds in the rest electrocardiogram, at low frequency, during the illness. At the follow-up examination one year later the rest P-Q time was 0.22 seconds and following work 0.16 seconds. It would seem that extreme normal variability was also present in this case, and the series cannot be interpreted as definitely pathological.

It was not felt to be possible to take a definite position in 6 cases, and the alterations have been recorded as equivocal. None of these patients have had subjective heart complaints. The following two cases are offered as an illustration of such equivocal variations:

A 15 year old boy (record no. 1228/49) displayed a variation of such a nature that it was impossible to say at which particular time there was an active A-V lesion, if at all:

Frequency:	95	65	60	55	55	85	75
P-Q time in seconds:	0.18	0.21	0.20	0.17	0.18	0.19	0.20

In addition he experienced fever and tachycardia the first week of illness and the electrocardiogram revealed a diphasic T_2. The changes are considered to be questionable evidence of myocarditis.

In a series of 7 electrocardiograms a 12 year old girl (record no. 1420/49) showed a variation between 0.14 and 0.19 seconds at low frequency. The higher values appeared in the three last electrocardiograms. At a follow-up examination the P-Q time was 0.18 seconds in rest and 0.16 seconds after work. This variation would seem to be questionable in nature.

A 10 year old girl (record no. 3305/46) had a variation of 0.04 seconds, but the longer P-Q time was observed at a low frequency; the shorter at a higher frequency. The following series was recorded and considered to be the possible result of extreme normal variability.

	During the Illness			After 1 yr.
Frequency:	75	100	90	75
P-Q time in seconds:	0.20	0.16	0.18	0.18

For the first 6 months after scarlatina she sometimes became very tired and experienced pain in the precordium after exertion. The pain was sharp and penetrating in character and caused her to bend over violently. This case has been listed as a probable myocarditis diagnosed on the basis of equivocal electrocardiographic findings associated with cardiac symptoms.

In this group it seems fitting to report the case of a patient whose P-Q variation seemed to lie within normal limits and whose electrocardiogram was also normal in other respects but who reported subjective heart difficulties. This was the case of a 3 year old boy (record no. 571/47) whose series was as follows:

Frequency:	95	85	80	105	110	110	120
P-Q time in seconds:	0.13	0.13	0.16	0.13	0.14	0.14	0.14

After 4 years the P-Q time was 0.14—0.16 seconds and decreased insignificantly after work.

In this case the range of variation, 0.03 seconds, as well as the upper value lie within normal limits. Considering the patient's growth, the upper value at the time of the follow-up examination is relatively lower than the one recorded during the illness. The patient's mother reported that after the illness he became very short of breath after running upstairs or playing. This was more marked than previously and more marked than in the other siblings. The clinical symptoms in this case would seem to indicate a myocarditis, but since the electrocardiographic variations were within normal limits the patient has not been listed among those whose electrocardiograms indicated myocarditis but has rather been listed with those patients with "symptomatic myocarditis" but apparently normal electrocardiograms (Chapter 17: Symtomatology, B. Symptoms).

In summation it may be said that in the case of small variations of as much as 0.04 and 0.05 seconds it is probably possible to decide whether it is a myocarditis or not only after a long series of electrocardiograms. The majority of these cases have not been classified as myocarditis.

CHAPTER 10

Changes in the T Waves

Introduction

The T waves are supposed to represent the repolarization process in the ventricles, and they are generally considered to be the most sensitive indicator of myocardial damage in the ventricles. The QRS complex on the other hand may remain unchanged in spite of considerable myocardial damage. Alterations in the T waves may also develop as a result of electrolyte disturbance such as hypopotassemia. Scarlatina itself has not been reported to result in such disturbances. Unequivocal T wave changes during the illness have therefore been considered as evidence of myocarditis in this study.

Normal Values

Adults: In the large groups of adult men reported by Graybiel et al. and Stewart and Manning T_1 was always positive although sometimes low. In lead II the T wave was negative twice and diphasic once in Graybiel's 1000 healthy test subjects and diphasic once in Stewart and Manning's group of 500. Statistical treatment of this material gives the following normal variation of the T wave: Lead I, from +1.0 to +5.0 mm. ($M \pm 2\sigma$) or from 0 to +6.0 mm. ($M \pm 3\sigma$); Lead II, from +1.0 to +6.6 mm. or from —0.4 to +8.0 mm. ($M \pm 2\sigma$, or $M \pm 3\sigma$). General experience agrees with these figures, and it would seem to be possible to establish the following criteria: T_1 and T_2 are normally positive. T_2 may be weakly negative, diphasic or isoelectric in isolated normal adults.

Children: In Mannheimer's study of normals T_1 and T_2 were always positive, and this would also be expected on the basis of the statistical calculations ($M \pm 3\sigma$). This agrees well with other studies on normal children (Maroney and Rantz 1950). Burnett et al. (1936) and Harris and Dawn (1948) also state that T_1 and T_2 are frequently higher in children than adults while T_3, on the contrary, is lower. It would seem, therefore, that a negative, isoelectric, or diphasic T_1 or T_2 is even more probably pathological in children than in adults.

Normal and Pathological Variations

T_1 and T_2 are frequently correlated with their R waves. They are definitely positive when the latter are high, while in the case of a right axis deviation T_1

may normally be almost completely flattened. Both variations from subject to subject as well as in the same subject are, however, considerable, especially in the case of children as Seham has stated.

However, in leads I and II low T waves as well as negative or isoelectric ones may indicate myocardial damage. They are seen as transitional forms in the development or regression of negative or isoelectric T waves. In other cases the alteration may consist only of a lowering of the T wave.

A low T wave can be evaluated only by a comparison with other electrocardiograms in the series from the same individual as Pardee (1947) and Sokolow (1948), among others, have noted. Roelsen (1941) reported T_1 and T_2 as flat when the waves were not more than 1 mm. in height or were lowered as much as 1 mm. However, it is difficult to establish definite limits for the interpretation. In most cases a T wave as low as 1 mm. in height is pathological. A lowering of the T wave of 1 mm. may in some cases, when the T wave is low to start with, indicate myocardial damage. In other cases lowerings of much greater magnitude may be within the normal range of variation when the T wave is ordinarily of high amplitude. It is thus the relative lowering as well as the relation between the R wave and the T wave that is important.

The shape of the T wave is also important (Alzamora-Castro 1946). Even normally high T waves may be an indication of myocardial damage if they have an abnormal form. As we know, the normal T wave is pointed but assymmetrical so that the descending slope is steeper than the ascending. No cases involving simply a change in form have been seen in the present study. When a lowering of the T wave has been associated with a double apex or a plateau shape it has been regarded as further support for a significant variation. This phenomenon has been observed in several cases.

Very high T waves may also be an indication of a pathological process (Byer et al. 1943, Bren and Zollner cit. Schmidt-Voigt, Dressler and Roesler 1947. Schmidt-Voigt 1949). In addition to being seen as a normal physiological phenomenon in markedly vagotonic subjects they are, according to these authors, seen in pathological condition such as infarcts, hypoxemia, uremia, and early pericarditis. Highly positive T waves have been observed a number of times in this study but always in electrocardiograms of the vagotonic type and in symptom-free patients and have not seemed to be pathological.

When the problem is one of demonstrating the presence of active myocardial damage as in this study, it must be remembered that aberrations from the normal may be the result of old lesions or uncommonly large constitutional variations. Thus Littman (1948) found abnormal T waves, which sometimes became normal as a result of certain physiological variations, in patients with apparently normal hearts. He therefore advised that supplementary studies be made in such cases—chest leads, recordings in various positions as well as at various times of the day, before and after meals, and over a prolonged period of observation. Such

supplementary studies have been made in this investigation in many cases with suspicious changes in the T wave. This is discussed further below.

Earlier Found Incidence in Scarlet Fever

In the literature dealing with scarlet fever myocarditis, the most commonly noted phenomena have been disturbances of A-V conduction or changes in the T wave. Differences between the various reports are chiefly the result of differences in interpretation. Thus Wickström (1932) reports a predominance of A-V conduction disturbances, but he is also rather generous in recording them. Roelsen (1941) reports the same incidence while with Nadrai (1941), S-T and T wave changes are commonest. Steinmann (1945) reports chiefly changes in the T waves in "Frühmyokarditis", while in "Spätmyokarditis" prolongations of the P-Q time predominate.

Own Interpretation

At present the author finds it impossible to set definite, numerical limits for the interpretation, which has in this study been based on the following rule. When a T wave has become strikingly low in relation to the R wave and there has been a marked lowering of the T wave with transition from or return to normally positive T waves, such a T wave has been considered "significantly flattened". If the variation cannot be ascribed to physiologically normal factors, the diagnosis of myocarditis has been made. This evaluation must be based partly on personal experience and vary somewhat from one author to another. All cases in which variations were even slightly questionable have been classified as equivocal and evaluated with consideration of the clinical course.

The changes usually involve T_1 and T_2, and these are considered together in the first part of the chapter. Changes in T_3 are of course seen at the same time in many cases. Alterations occurring mainly in T_3 will be dealt with in a special section at the end of the chapter.

The Differential Diagnosis between Physiologically Normal and Pathological Variations in the T Wave

I. *Variations accompanying sympatheticotonic states*

The state of increased sympatheticotonia is of interest in this connection since flattening and possibly inverting of the T waves may result and thus cause difficulties in the differential diagnosis with myocarditis. This condition and our diagnostic procedures have been discussed earlier (pp. 27—31). The use and interpretation of the tests will be illustrated in this chapter.

The mechanism is so complex that variations of a vegetative nature may be of varying type even in the same patient. For example, a 15 year old girl (record no. 1274/49) showed a negative T_2 and T_3, a typical sympatheticotonic electro-

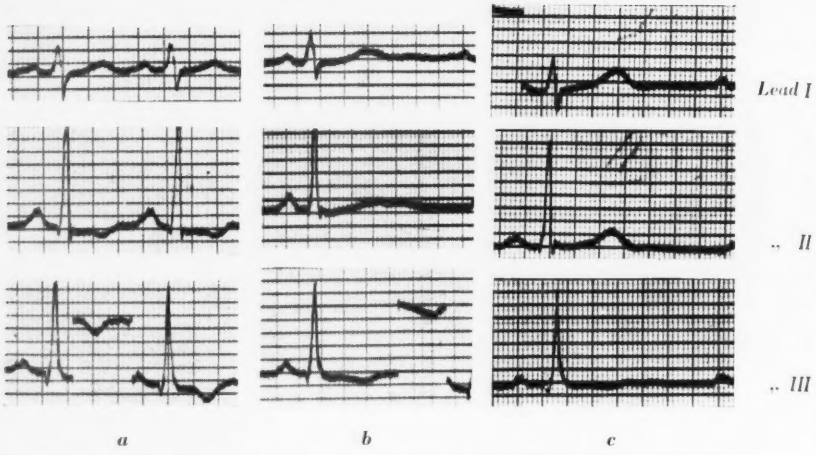


Fig. 17. *Reproduceable variations in the T waves.* (Record no. 1274/49.)

a. Changes of the sympatheticotonic type during initial period of fever at a frequency of 135. High pointed P waves are seen while T_1 is positive, T_2 is negative to diphasic, and T_3 negative.

b. Changes of the type seen when adrenalin is administered experimentally. The frequency is 85. Note high-pointed P waves. There is a marked positive after-potential in lead II, which is responsible for the fact that T_2 appears to have a double apex. T_1 and T_2 are positive and T_3 is negative.

c. A normal electrocardiogram with a frequency of approximately 75. The P waves are positive although lower than in a. and b. while T_1 and T_2 are highly positive and T_3 diphasic.

cardiogram, in her first recording (figure 17 a), at which time she had a fever and a pulse rate of 135. In the second electrocardiogram (figure 17 b) the frequency was lower and the T waves were elevated, but T_2 seemed to have two peaks. This double peak was probably only apparent, the result of a marked positive after-potential such as may be seen after experimentally increasing the adrenalin content of the blood (Sjöstrand. 1951). Figure 17 c shows the patient's third electrocardiogram, which was entirely normal. At the time of the follow-up examination one year later marked lability of the T wave and variations similar to those previously observed were seen following inhalation of amyl nitrite. These electrocardiographic changes have consequently not been regarded as definitely indicative of heart disease. The patient has been free of symptoms.

a) The Amyl Nitrite Test

As mentioned earlier we have frequently used the amyl nitrite test as a procedure to determine whether a lowering of the T wave suspected to be due to a sympatheticotonia with an increase in frequency can be reproduced at a similar frequency.

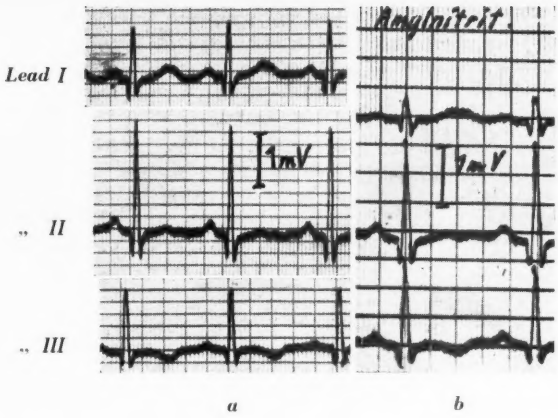


Fig. 18. *Amyl nitrite test in a case with flattening of the T waves.* (Record no. 4482/47, leads I—III.)

a. During the initial fever the frequency is 120. T_2 is flattened with a double apex while T_3 is deeply negative.
b. Amyl nitrite test at a follow-up examination 3 years later. At a frequency of 120 the flattening of T_2 is fully reproduced.

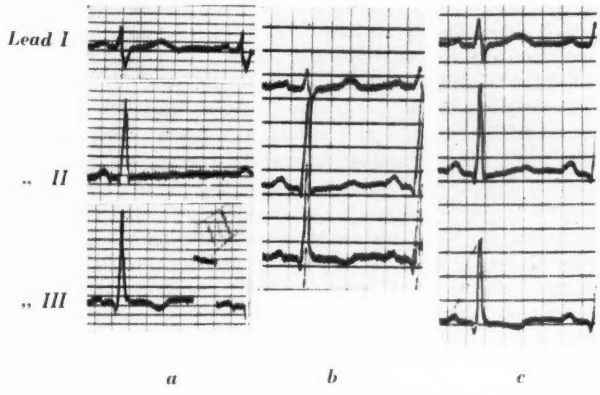


Fig. 19. *Amyl nitrite test in a case with flattening of the T waves.* (Record no. 1104/49.)

a. Recording during first week of illness. At a frequency of 70—85 T_2 is isoelectric indicating a myocarditis.
b. From the fifth week of illness. Frequency 100 with T_2 flattened and T_3 negative. A sympatheticotonia seems to be the cause.
c. A section of a recording taken after inhalation of amyl nitrite at the time of follow-up examination. The same changes as seen in b. above appear. The change seen in a. could not be reproduced.

In a number of cases, such as the following one, a suspected myocarditis has been less probable on the basis of the amyl nitrite test. This was a 3 year old girl (record no. 4482/47) who displayed an obviously flattened T_2 in her first electrocardiogram, which was recorded on the fourth day of illness in the presence of a fever (figure 18 a). T_2 was later significantly elevated. A follow-up examination was done using the amyl nitrite test. T_2 was low in rest, and after amyl nitrite it was definitely lowered with two peaks (figure 18 b). This was in excess of the findings in the earlier suspiciously pathological electrocardiogram. The alteration would therefore seem to have been the result of a sympatheticotonia rather than a myocarditis.

A 9 year old girl (record no. 1104/49) showed an isoelectric T_2 and a negative T_3 at a frequency of 75—100 in her first electrocardiogram (figure 19 a). In the fourth recording T_2 was flattened and T_3 negative at a rate of 100 (figure 19 b). In the remaining electrocardiograms T_2 was highly positive and T_3 positive or isoelectric. At a follow-up one year later the changes in the fourth electrocardiogram, but not the first, were reproducible with amyl nitrite (figure 19 c). Apparently there was a myocarditis present at the first examination, while the changes in figure 19 b may have been due to vegetative factors.

b) The Ergotamine Test

In the presence of persistent alterations in the electrocardiogram suspected of being due to a sympatheticotonia the ergotamine test has been made in some cases. These have been interpreted with caution, however (see page 30).

In the following case the ergotamine test supported a diagnosis of constitutional sympatheticotonia. This patient was an 8 year old girl (record 1178/49). During an observation period of one year she usually had rather high frequency which was associated with a flattening of T_1 so that it was only suggestively positive while T_2 was isoelectric or weakly negative. The S-T segments were lowered, $S\text{-}T_4$ as much as 3 mm. (figure 20 a). As a rule there was an elevation of the T wave following exertion, and in the ergotamine test the lowered frequency was associated with very clear elevation of the T waves and the S-T segment (figure 20 b). Following her return home from the hospital the patient was a little tired and complained of tingling sensations in her side. Judging from the description these complaints may have been muscular. The patient is alert and lively; and she blushes readily, further evidence of vascular lability. The strongest evidence of the constitutional sympatheticotonic nature of the condition in this case, however, is the fact that the alteration remained unchanged throughout the entire period of observation without any associated reduction in physical capacity. Similar cases will be reported in the chapter dealing with S-T changes.

Also in the following case (record no. 3014/48) the electrocardiogram was practically normalized during the ergotamine test although it was felt that there was probably a myocarditis. This was an 8 year old girl who showed alterations of T_2 from definitely positive to slightly negative in her series of electrocardiograms. T_1 was always positive and T_3 negative. In one of her earlier electrocardiograms, at a frequency of 85, T_2 was clearly positive (figure 21 a) although P_2 was relatively high and pointed. By comparison, a later electrocardiogram in figure 21 b seems to be abnormal. In this instance, at a frequency of 115, T_2 was negative and there was a suggestion of a rounded elevation of the S-T segment. The isoelectric T_2 in a later electrocardiogram (figure 21 c) at a frequency of 90 would also seem to be abnormal, especially compared with figure a. After ergotamine, however, T_2 became positive at a frequency of 80. As pointed out previously (page 31), such a normalization of T_2 may be an expression of functional variability or may even be simulated. This negative and isoelectric T_2 is so different from the usual finding in normal children according to the literature and in the patient's series of electrocardiograms that the change has been classified as pathological in spite of the ergotamine test. Unfortunately it has not been possible to contact this patient for follow-up studies. These would have been very

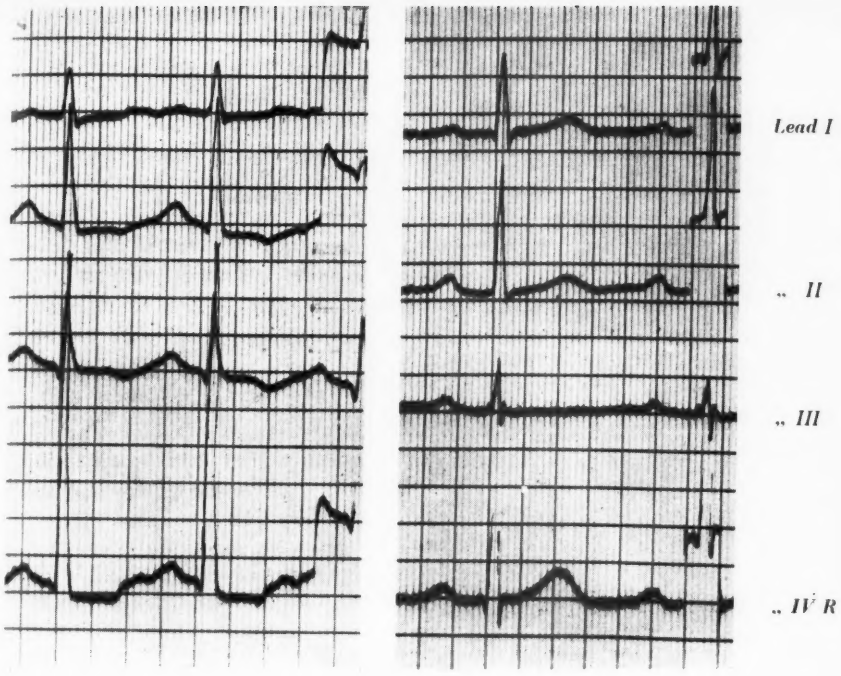


Fig. 20. *Persistent depression of the S-T segment and the T wave with an ergotamine test supporting a diagnosis of sympatheticotonia.* (Record no. 1178/49, leads I—III and IV R.)

a. A marked positive after-potential is seen in this recording taken during rest with a frequency of 150. The S-T segments are depressed in all leads, most markedly in lead IVR. T_1 is diphasic while T_2, T_3 and T_4 are negative.

b. Recording on the same occasion following injection of 0.25 mg. "Gynergen". The frequency is 90, no after-potential is present, the S-T segments are isoelectric as is T_3. T_1, T_2, and T_4 are positive.

worthwhile and could have clarified the diagnosis. At the time the alteration developed the patient complained of fatigue, vertigo, and headaches. However, these may have been simply due to orthostatic difficulties.

c) Prolonged observation

A long period of observation would seem to be of considerable value in the differential diagnosis of alterations in the T wave which may be due to a sympatheticotonia (see also page 31). In this study, even severe changes in the T wave resulting from definite scarlatina myocarditis have been entirely normalized. For this reason a persistent alteration must be suspected of being due to a constitutional aberration or an old lesion. As an example see figure 20.

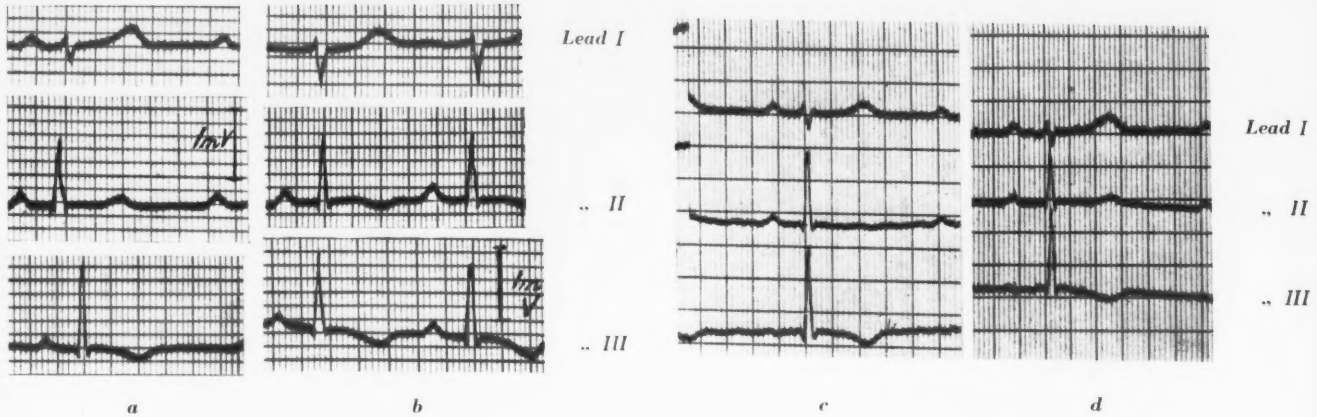


Fig. 21. *Flattening of the T waves probably due to myocarditis in spite of normalization following an ergotamine test.* (Record no. 3014/48, leads I—III.)

a. A normal electrocardiogram with a frequency of 85. T_1 and T_2 are distinctly positive, T_3 is negative.

b. Frequency 115. T_1 is positive, T_2 and T_3 are negative.

c. Frequency 90. T_1 is positive, T_2 is isoelectric and T_3 negative.

d. Recording after 0.25 mg. Gynergen on the same occasion as figure c. The frequency is 80 and T_1 and T_2 are positive.

Cases with possibly sympatheticotonic changes in the T waves

Flattening of the T wave of more marked degree, sometimes coincident with S-T depression, in which the alterations may be related to an increase in frequency — increased sympatheticotonus and adrenalin in the blood — has been observed in 35 cases. Six patients have displayed changes predominantly of T_2.

In the presence of such alterations, which it seems might be due to vegetative factors, it is of course very important to consider the clinical picture. The possibility of myocardial damage manifesting itself in tachycardia can not be ruled out. Therefore, if clinical symptoms have been present in addition to such changes in the electrocardiogram a diagnosis of myocarditis has been felt to be justified. These patients with equivocal changes in the electrocardiogram plus symptoms have, however, been assigned to a special group of probable myocarditis (page 23).

This has been the situation with 3 of the patients with flattening of T_2 and 3 patients whose chief change consisted of a negative T_3. A short resumé of these patients is offered below.

A 15 year old girl (record no. 700/48) was admitted in the desquamation stage of scarlet fever one month subsequent to experiencing sore throat with joint complaints. Her antistreptolysin titer was high — up to 1 to 2200. Her pulse rate was 120, and T_2 was markedly flattened and T_3 negative. Three days later at a frequency of 75, T_2 was highly positive. At the time of a follow-up examination three years later the previously observed changes in the electrocardiogram could be reproduced by amyl nitrite. They would therefore seem to have been related to the high frequency. The patient reported, however, that immediately after returning home from the hospital she had suffered from marked dyspnea. She subsequently returned to her usual state, and it would therefore seem likely that there had been some myocardial damage.

In a number of her recordings a 5 year old girl (record no. 3752/48) showed a high frequency, 100 to 120, and flattening of T_2. In addition, some of her other recordings showed a depression of S-T in leads II and III. The changes were of the type usually seen with sympatheticotonia, and it was possible to reproduce them at the follow-up examination. Furthermore, the T wave in CR_4 was negative to diphasic in the first electrocardiogram but positive in later ones. However, at one of the check-ups she was reported to have been tired and fretful, to have spontaneously lain down in the middle of the day, to have been unable to walk up hills, and to have complained of pain in the left side. These clinical findings would seem to indicate a myocarditis.

One 7 year old girl (record no. 4547/48) was found to have a practically isoelectric T_2 with a tachycardia of 135 at one of her check-ups. At this time, as well as in preceding and succeeding recordings, there were premature auricular extra systoles. The flattening of T_2 may have been related to the frequency, but following the scarlet fever the patient was very tired and dyspneic. In association with the electrocardiographic changes this makes a diagnosis of myocarditis seem probable.

No less than 3 of the 6 patients (record nos. 3748/47, 3889/47, and 3059/48) whose T_3 changed from positive to negative with tachycardia complained of dyspnea when going up stairs or hills in the interval just following the illness. It would therefore seem that these cases may also have been suffering from myocarditis. The two first cases had also shown depressions of the S-T segment.

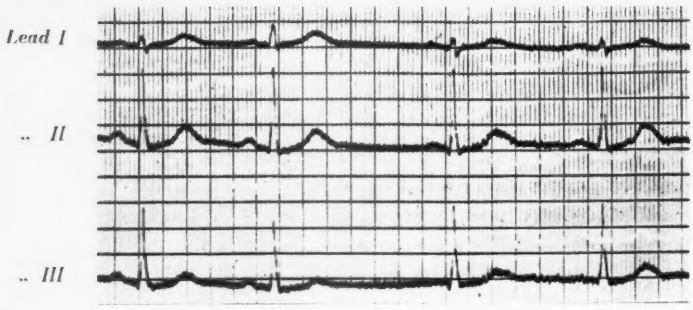


Fig. 22. *Spontaneous variations of the axis.* (Record no. 2174/49.)

The recording shows leads I—III immediately after work with changes in QRS and T which are probably caused by respiration. T_1 is lowered in association with a shifting of the ventricular axis to the right. Seemingly independent of the axis shift a wandering of the pacemaker appears with sinus rhythm in the first and second beats and perisinus rhythm in the third and fourth beats.

II. *Variations Accompanying Changes of Position*

Concurrent changes in the T waves and the ventricular complexes, as for example flattening of T_1, with shifting of the electrical axis to the right, or depression of T_3 with an axis shift to the left, should, as previously noted (p. 33—35), be interpreted in the light of the variations which occur in response to changes of body position following recovery. In the case of children, the follow-up examination should be made before more than a year or so has passed, since alterations due to growth may otherwise make the comparison difficult. It is at times possible to observe these so-called "positional" changes occurring in a single recording as a result of the respiratory movements. This may be seen in figure 22 which was recorded immediately after work from a 5 year old boy (record no. 2174/49) and shows how a flattening of T_1 accompanies a shift of the electrical axis to the right. A small shift in the pacemaker was also observed, but it was apparently unrelated to the form of the QRS complex.

Flattening of T_1 of marked degree coincident with a shift of the axis to the right was observed in 6 cases, in 2 of whom the variation was reproducible at the time of follow-up examination.

In the case of an 8 year old boy (record no. 2920/48) it may be seen how greatly the T wave may vary in response to changes of position. During the illness he showed a deepening of S_1 coincident with a suspicious flattening of T_1. In order to study the effects of positional changes on T_1, electrocardiograms were taken in various positions at the time of the follow-up examination 2 years later. T_1 was found to be highly positive in the supine position (figure 23 a). In the left lateral position the QRS complex was altered with a deepening of S_1. Simultaneously a marked change occurred in T_1 which became isoelectric (figure 23 b). The coincident increase in pulse frequency may also have been a contributing factor. This electro-

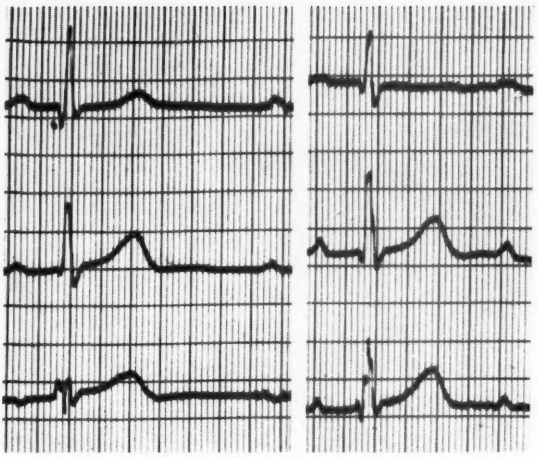


Fig. 23. *Considerable alterations in T_1 in variations of the horizontal position.* (Record no. 2920/48, leads I—III taken at the follow-up examination.)

a. In the supine position T_1 is distinctly positive. There are notchings in R_1 and R_2, and QRS_3 is splintered.

b. In the left lateral position the ventricular axis shifts to the right and T_1 is isoelectric.

cardiogram is obviously pathological in character. The variation demonstrates how a change may make its appearance in only a certain position. The observed flattening of T_1 during the scarlatina may have been due to positional changes and is a doubtful sign of acute myocarditis. The boy had been symptomfree.

In the third case (record no. 3995/47) the earlier types of QRS appeared at the follow-up examination, but the amplitude of QRS_1 was diminished and the flattening of T_1 was less marked (figure 24). This might have been due to the fact that three years had elapsed since the illness, and the T wave over the right ventricle had probably increased in height during this time. It is worthy of note, however, that a very clear systolic murmur was heard at the time that the axis shift to the right was observed. This murmur subsequently diminished in strength and changed in quality. The x-ray of the heart at the time was normal. There had been no symptoms and the case is listed as questionably pathological. In another two cases flattening of T_1 was not observed in the same degree at the time of the follow-up examination, but the interval was too long for a dependable interpretation. These cases are considered to show equivocal changes. Since one of these patients (record no. 645/48) suffered with noticeable dyspnea after the scarlet fever he has been included in the probable myocarditis group with equivocal electrocardiographic changes plus cardiac symptoms. This method would probably have given more valuable information had it been used from the beginning of the investigation, but the author did not use it until 2 years had elapsed.

In still another case (record no. 3474/48) a flattening of T_1 was seen in conjunction with a diminished amplitude. This low amplitude was also observed in a later electrocardiogram although at this time T_1 was clearly positive. At the time of the follow-up examination T_1 in the left lateral position was markedly flattened, but the ventricular complex's electrical axis was then displaced even further to the right than previously. The patient

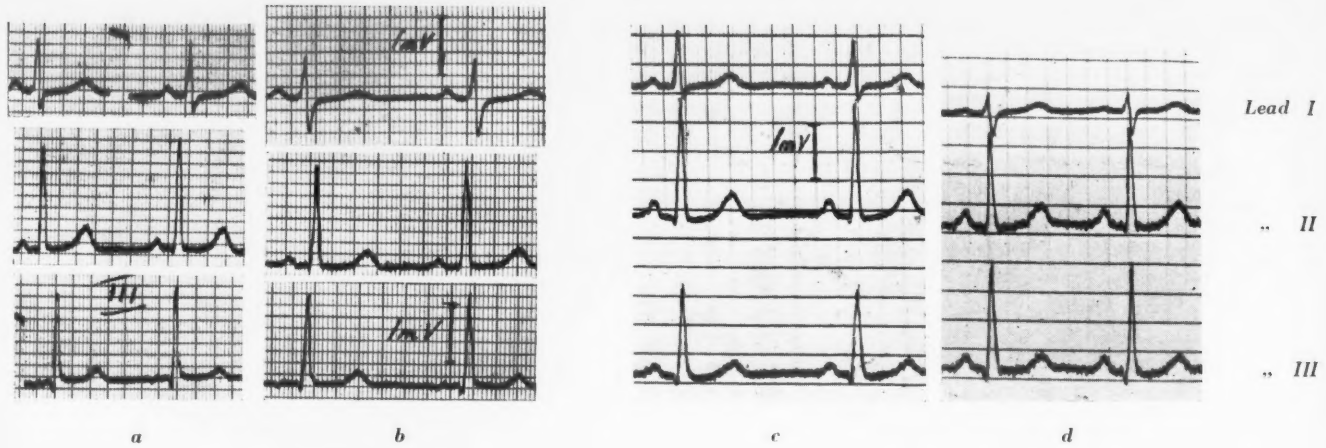


Fig. 24. *Deviation of the ventricular axis to the right in association with flattening of T_1 not wholly reproduceable in various horizontal positions.* (Record no. 3995/47, leads I—III.)

a. The common type of electrocardiogram taken during the disease.

b. Recording in the fifth week of the disease showing deviation to the right of the ventricular axis and lowering of T_1.

(Figures c and d are recorded at the follow-up examination 3 years later.)

c. In the right lateral position the electrocardiogram has the same appearance as in figure a.

d. In the left lateral position deviation of the ventricular axis to the right occurs. However, the amplitude in lead I is lower than in figure b, and T_1 is less flattened.

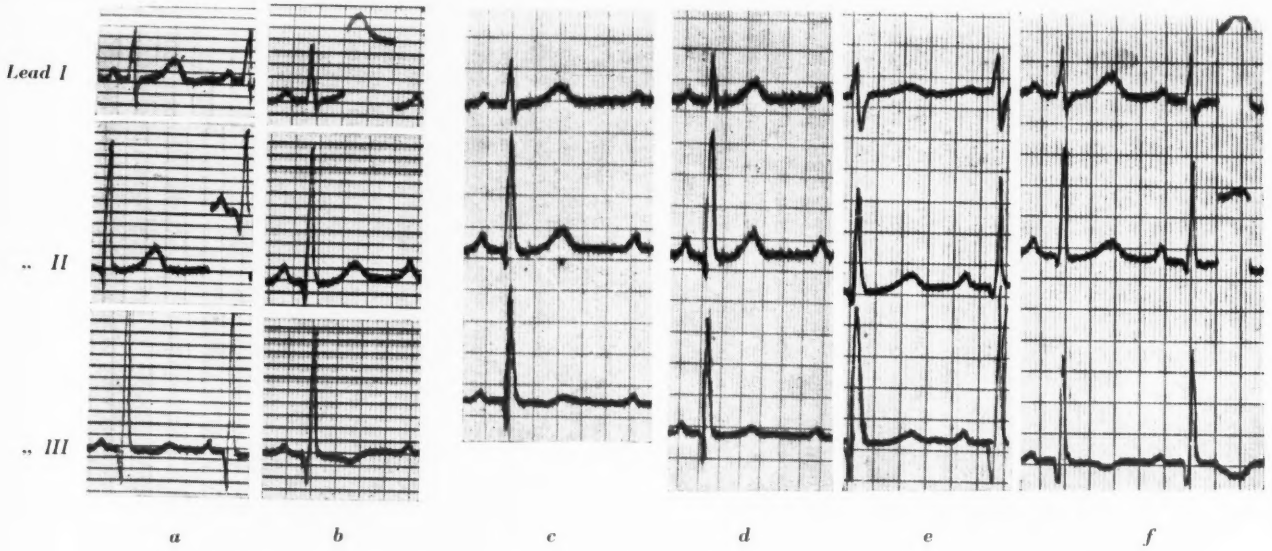


Fig. 25. *Variations in T_3 reproduceable in various horizontal positions.* (Record no. 355/49, leads I—III.)

The recordings in figures a and b are taken during the disease, in c to f at the follow-up examination 1 year later.

a. T_3 is positive, and the axis deviates slightly to the right.

b. T_3 is negative in association with a decreasing of S_1.

c. In the supine position T_3 is positive and has the same appearance as in figure a.

d. In the right lateral position T_3 is positive and S_1 has decreased.

e. In the left lateral position T_1 is lowered in association with deviation of the electrical axis to the right; T_3 is positive.

f. In the prone position T_3 is negative and the recording is the same as in figure b.

has been free of symptoms, and this has been recorded as an equivocal change. In addition this girl had a consistent intraventricular conduction disturbance in the right ventricle. This, however, remained unaltered during the one and a half year of observation and would seem to be unrelated to the scarlet fever.

In some cases with large spontaneous changes of T_3, recordings taken in various positions have indicated that the changes might have been due to positional alterations.

In the case of a 5 year old girl (record no. 355/49) T_3 changed from negative to positive (figures 25 a and b), and when electrocardiograms were recorded in different positions at follow-up examination definite changes in T_3 and also in T_1 were observed (figures 25 c, d, e, and f). In the supine position T_3 was positive; in the right and left lateral position, positive; and in the prone position, negative. T_1 was notably flattened in the left lateral position. The ventricular complexes also varied as may be seen most clearly in lead I. The series as a whole demonstrates nicely how variations in position effect different elements of the electrocardiogram. A comparison between the left and right lateral positions is illuminating. As may be noted, changes in both the ventricular complex and the T wave are here most predominent in lead I. From the right lateral position to the prone, however, the chief changes are in T_3.

Other cases have been observed in which changes of the QRS complex have been associated with flattening of the T wave where changes of position reproduced the QRS changes but not those of the T wave. These would seem to have been due to myocardial damage, and in such cases the changes have often not been of the type which seems to be related to or caused by variations of position. Whether the myocardial lesion also caused the changes in the QRS complex is difficult to decide however.

Again in other cases depression of the T waves and S-T segments has been seen with axis variation apparently unrelated to myocardial damage since the same type of QRS was observed with both normal and with abnormal T waves.

For example, an 8 year old girl (record no. 2628/49) displayed a suggestion of right axis shift in her first and third electrocardiograms (figure 26 a and c). In addition, recording number one showed a diphasic T_2, a negative T_3, and tachycardia. In the intermediate and later electrocardiograms R_1 was higher and S_1 was lower (figure 26 b), and at the follow-up examination two years later the QRS changes could be reproduced in various positions (figure 26 d and e). The changes in T_2 and T_3, however, did not reappear either in response to positional variations or inhalation of amyl nitrite (figure 26 f) and would thus seem to have been a result of a myocarditis. The changes in the QRS complex, which may have been due to position, thus occurred independently of the depression of T_2 and T_3 resulting from the myocarditis in this case.

Artefacts

Attention must also be paid to the possibility of T wave aberrations resulting from faulty electrocardiographic apparatus. In this study such alterations have been observed on two occasions. They were of similar type and occurred mainly or entirely in lead I. A change in the form of the ventricular complex occurred involving a lowered R wave, a deeper S wave, and simultaneous appearance of a flat or diphasic T wave with a negative after-potential. Sometimes there was

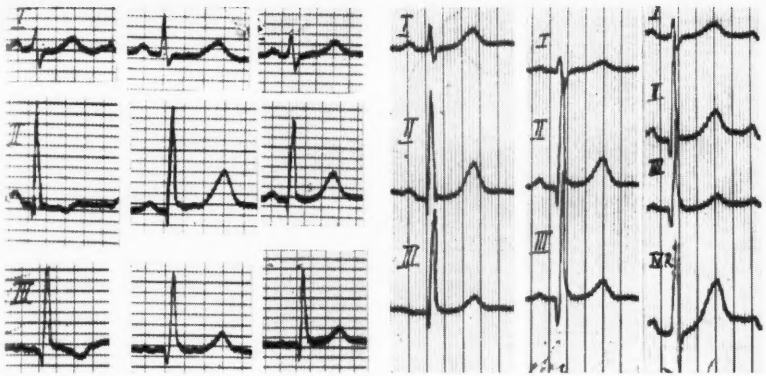


Fig. 26. *Variations in the axis seemingly independent of myocarditical changes in the T waves.* (Record no. 2628/49.)

a., b. and c. are taken during the disease. In a. and c. the axis deviates to the right in comparison to b. In addition, T_2 and T_3 are inverted in figure a., indicating a myocarditis. d., e. and f. are recorded at the follow-up examination. Figure d. is taken in the supine position; e. is recorded in the left lateral position which causes a shift to the right of the axis as in figures a. and c; f. is a section taken after inhalation of amyl nitrite. The inverting of T_2 and T_3 previously observed is not reproduced either in various positions or following amyl nitrite.

an elevation of the S-T segment and flattening or sometimes inversion of the P waves. This type of distortion is described by Scherf and Boyde (1945) with the string galvanometer and by Schenetten, (1947), and Moe, (1948), and is said to be probably a result of faulty wiring with polarization. Because of the importance of recognizing this it would seem to be worth mentioning at this time.

Sometimes the distortion is of such peculiar form that an artefact is suggested immediately, but in other cases they may be extremely difficult to differentiate from myocardial lesions or changes due to position. Another factor which makes artefacts deceptive is the fact that they may appear in only a part of the patients examined during the same period and may occur in the same patient several times in succession. What called it to our attention was the fact that during a certain period several patients suddenly developed "myocardial lesions" with the same general distortions of the electrocardiogram. Figure 27 shows two electrocardiograms from the first period from the same patient (record no. 991/48). These were recorded in immediate succession, a) with the faulty and b) with a correctly functioning apparatus. The faulty apparatus was a new one and one of the first of a new type. A leakage occurred in a complicated mecha-

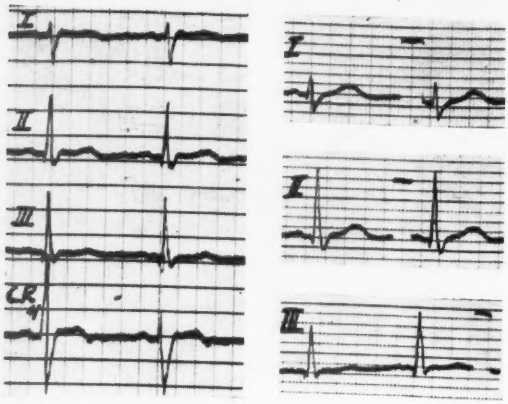


Fig. 27. *An artefact simulating myocardial damage.* (Record no. 991/48.)

a. Leads I—III and CR_1 recorded with a faulty apparatus, showing right axis deviation and flattening of T_1 and T_2.

b. Leads I—III taken immediately after with a correctly functioning apparatus. There is a slight right axis-deviation, and T_1 and T_2 are distinctly positive.

nism, but this occurred only between long intervals of normal operation and only with the use of the thoracic leads. Such errors have been eliminated by rebuilding the apparatus. The nature of this artefact was entirely clarified and could be recognized easily. At the same time we had another apparatus in use and frequent electrocardiograms were recorded from the majority of the patients. Since the artefact only appeared sporadically and only when the chest leads were used, the patients from this period have been allowed to remain in the material.

The second apparatus failure was first noticed on reviewing the records. Figure 28, which was recorded at this time, shows both a correctly recorded

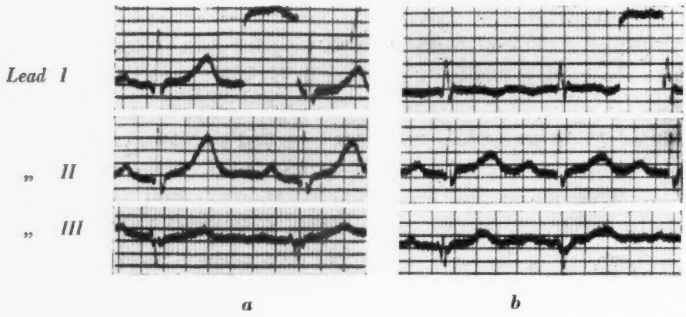


Fig. 28. *A peculiar distortion of the electrocardiogram caused by faulty apparatus.* (Record no. 2681/49.)

a. A correctly recorded electrocardiogram.

b. An electrocardiogram taken with a faulty apparatus. P_1 and T_1 are inverted, and the amplitude of R_1 is decreased.

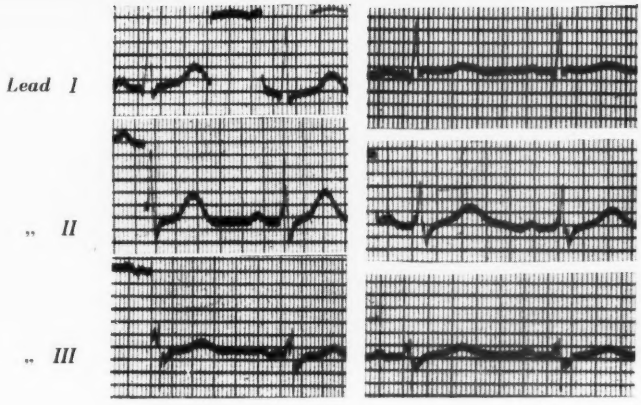


Fig. 29. *Alteration in an electrocardiogram — possibly an artefact.* (Record no. 2538/49, leads I—III.)

a. The patient's normal electrocardiogram.

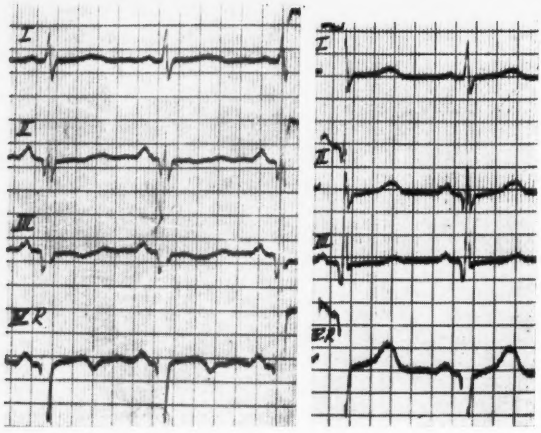
b. Lowering of T_1 and elevation of $S\text{-}T_1$, which seem to indicate myocardial damage. However, in view of the findings in figure 28 b the alteration might be an artefact.

electrocardiogram and one of the two faulty ones from the same patient (record no. 2681/49). In this case the aberration was very striking and there was a marked discrepancy between the leads. In another electrocardiogram, however, (figures 29 a and b; record no. 2538/49) recorded during the same period there is an alteration which would appear to fit the picture of a myocarditis but which if considered in the light of other distortions might very well be an artefact. This artefact was observed in cases who were examined during the last month of the study, and since the evaluation of these electrocardiograms would be highly doubtful all patients from this period have been dropped from the material. Some of them have, however, been mentioned in the text when correctly recorded electrocardiograms illustrated certain types of variations.

Other Diagnostic Procedures

The work electrocardiogram

The earliest and still most important use of the work electrocardiogram is in suspected coronary disease with the associated changes in the terminal deflection. However, myocarditis may cause similar changes in the electrocardiogram, and it appears that these changes may also be accentuated or in certain cases first manifested in the work electrocardiogram. (See Klemola for bibliography.) On the other hand, Scherf (1947) feels that the work electrocardiogram is positive only in the presence of coronary disease. Since pathological sections have shown



a *b*

Fig. 30. *A work electrocardiogram, probably pathological, taken during convalescence, and a normal work electrocardiogram after recovery.* (Record no. 88/49, leads I—III and IV R 4 minutes after work.)

a. In the fifth week the frequency is 105. T_1 is flattened, T_2 isoelectric to slightly positive, T_3 slightly negative, T_4 negative.

b. Three months later the frequency is 100. The T waves are distinctly positive.

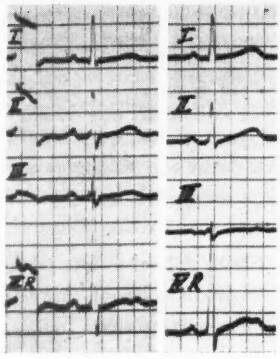
that infectious diseases, especially rheumatic fever, may also be associated with coronary lesions (de Brux 1948, Plotz 1948), the use of the work electrocardiogram in infectious diseases would seem to be justifiable in any case.

In the cases with changes in the T waves and the S-T segment indicative of an active process the work test has not been done. It has been carried out after normalization has occurred or when the changes were only of a questionable character. The work test reaction has been interpreted chiefly on the basis of the criteria established by Biörck (1946), Lindgren (1946), Scherf (1947), and Sjöstrand (1951). Inverted, diphasic, or isoelectric T waves have been considered as abnormal as have depressions of the S-T segment of 1.5 mm. or more. The shape of the S-T segment is also important. Less stern criteria, such as depressions of 0.5 mm. and simple flattenings of the T wave, would seem to include even some normal cases. However, when there is only a small increase in frequency, if any, even minor alterations are probably an indication of myocardial damage.

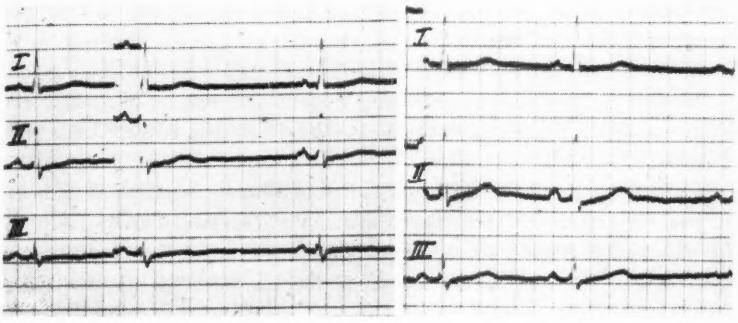
In this study in association with a suspected myocarditis, changes in the work electrocardiogram have sometimes been observed which did not reappear after recovery. Although the strictest criteria for pathological work electrocardiogram have not always been completely met in these cases, we have still found the work reaction to support a diagnosis of myocarditis as for example in the following cases.

Fig. 31. *Aberrent work electrocardiogram in association with a probable myocarditis.* (Record no. 3857/48.)

a. Leads I—III and IVR recorded at rest in the fifth week. The frequency is 85, and T_1 is flattened.
b. Leads I—III and IVR recorded at rest one week later. The frequency is 70, and T_1 is distinctly positive. In lead II the S-T segment and the ascending limb of the T wave form a straight line.
c. Leads I—III recorded immediately after work at the same time as b. There is sinus arrhythmia; T_1 and T_2 are lowered.
d. Leads I—III immediately after work one month later, showing sinus arrhythmia; T_1 and T_2 are distinctly positive.



a *b*



c *d*

A 5 year old boy (record no. 88/49) displayed a highly positive T_1 and T_2 during his stay in hospital. At a check-up in the fifth week the T waves were flattened without an increase in frequency. The sedimentation rate was also increased, and the patient had experienced a slight fever some days previously. Figure 30 a shows a work electrocardiogram from this patient recorded at the next check-up at a 4 minute interval after work. The T waves are markedly flattened; and T_4, which apparently is recorded partly from the left ventricle, is negative. Three months later T_1 and T_2 as well as T_4 were positive both in rest and after work (figure 30 b). The same state prevailed at a follow-up examination one and a half years later. The patient's mother stated that immediately after his scarlet fever he was tired and listless, became short of breath on hills, and that his disposition was fretful and aggressive. He has been grouped as "probable myocarditis with symptoms".

An 8 year old boy (record no. 3857/48) had a highly positive T_1 and T_2 in his first recordings. At check-up in the fifth week T_1 was flattened. The frequency had increased somewhat, however, and the P waves were pointed, indicating an increase in sympathetic tone (figure 31 a). In another recording one week later the T waves were again clearly positive at a lower frequency. In lead II, however, the S-T segment and the ascending limb of the T wave formed a straight line (see figure 31 b) which is pathological according to Blackman and Hamilton (1948). Immediately after work T_1 and T_2 became flattened despite a low frequency and without a shift of the electrical axis (figure 31 c). This change is on

the borderline of permissible variation, but it is probably pathological in this case, especially since a work electrocardiogram one month later was entirely normal with a highly positive T_1 and T_2 (figure 31 d). For a time just after his illness the patient's condition was poor, he had no energy, and he would spontaneously lie down during play.

In still another case (record no. 1858/49) an 8 year old girl with flattening of T_1 and T_2 during rest, the T waves were entirely isoelectric following work during the period in which the change was evident, but at subsequent examinations this was no longer true.

Precordial and Unipolar Extremity Leads

With regard to adults, the standards established by Goldberger, Wilson, and Grewin have been followed. The interpretation of the precordial leads in children is more complicated by the progressive transition from the child's electrocardiogram to that of the adult. The T wave in children is discussed by a number of authors, among others Master et al. 1936, Lepeschkin 1938, Asch 1945, Battro and Mendy 1946, Goldberger 1946, and Littman 1947. In infants the T waves are negative in all of Wilson's positions 1 through 6. They become positive successively from left to right, and in the adult only lead 1, and seldom lead 2, remains negative. By the age of 12 the T wave should have become positive in position 4 (Suarez and Suarez 1946, Kuskin and Brochman 1949), and the adult type appears appreciably earlier in the R leads than in the F leads (Littman 1947).

While a negative T_4 in a 12 year old child must be regarded as pathological, in younger children its presence is difficult to evaluate. Variations in T_4 also have a questionable significance especially since Littman observed such changes even in healthy children. Variations in the placement of the electrodes and spontaneous shifts in the electrical axis of the heart may cause them due to changes in the relationship of the electrodes to the heart. A negative T wave in a Wilson lead corresponding to the left ventricle and a qR(s) ventricular pattern would, however, seem to be a definitely pathological sign even in small children (Goldberger 1946).

The precordial leads have been used to supplement the examination in pathological and questionable cases during the later years. They would seem to offer additional information by verifying and localizing suspicious lesions and by demonstrating the presence of a persistent change after the standard leads have been normalized. The following two cases are given to illustrate the value of these leads.

The first one is that of the 5 year old boy (record no. 88/49) reported earlier who developed a flattening of T_1 and T_2. At the same time T in IV R was negative (figure 30 a) although this lead showed a qRS pattern. The change persisted in a few subsequent electrocardiograms, but the T wave later became definitely positive (figure 30 b) and was still positive at a follow-up examination one year later. Since the ventricular complex was unaltered it would seem that the electrodes were placed in the same position. The change is regarded as supporting a diagnosis of myocarditis.

The other case was that of a 9 year old girl (record no. 883/49). She first developed a streptococcus sore throat and shortly thereafter the measles. She was admitted one month after the original onset, when the presence of scarlatina desquamation was established. She also had had a transient arthritis, and there was a prolongation of the P-Q time in the electrocardiogram up to 0.30 seconds. It is likely that a rheumatic type of infection had developed in this case. The clinical course during the first two or three weeks was rather serious with evidence of cardiac decompensation — dilation of the heart, enlargement of the liver, and cyanosis. With regard to the examination of the heart, a rough systolic murmur was heard at the base at the time of admission. Later there was a prolonged systolic murmur at the apex which, however, disappeared. X-rays of the heart verified cardiac dilatation. The following series of heart volumes (cc./M.^{2} of body surface) was obtained: On admission — 392 cc./M.^{2}, after 2 weeks — 268 cc./M.^{2}, after 6 weeks — 321 cc./M.^{2}, after 3½ months 260 cc./M.^{2}, after 7 months — 321 cc./M.^{2}, after 10 months — 291 cc./M.^{2}, and after 1 year and 3 months — 266 cc./M.^{2}.

Within 10 days the P-Q time had decreased to 0.19 seconds, and the standard leads were practically normal. However, the precordial and unipolar extremity leads had also shown alterations, and these remained much longer. The most persistent change was a lowering of the S-T segment in aVR amounting to 2 mm. This decreased to 0.5 mm. after 6 months. It was only after another 6 months that S-T was isoelectric and both the resting and work electrocardiogram normal. For a time immediately following the illness the patient was markedly dyspneic even after minor exertion. There was gradual improvement so that at the time of the follow-up examination the patient was capable of moderate exercise, but she became very short of breath with more strenuous exertion. Thus in this case alterations could be seen longer in the unipolar leads than in the standard leads, and these persistent changes were in agreement with the clinical picture.

With regard to the increased information to be obtained from the precordial leads see also case no. 4682/47 page 129, figure 35.

The Author's Cases with Changes in T_1 and T_2 Ascribed to Myocarditis

Classification

The cases have been divided into different groups according to the degree of alteration of the T wave in the electrocardiogram. Group A comprises the cases with negative T waves; group B, those with isoelectric T waves; group C diphasic; and group D those with marked lowering of T_1 and/or T_2. In addition, questionable flattenings, associated with subjective heart complaints suggestive of acute cardiac disease, have been gathered in group E. In tables 6 and 7 the onset and duration of the changes within the different groups are indicated as well as the age distribution and treatment series.

TABLE 6

Changes in the T Waves in lead I and II

Distribution of the cases with regard to groups (page 123), onset, and duration.

Group	Number	Onset				Duration							
		1st wk.	2nd—3rd wk.	4th—5th wk.	After 5th wk.	Seen once	1 wk.	2—4 wk.	5—8 wk.	9 wk.—5 mo.	6—12 mo.	> 1 yr.	Unknown
A	8	3	5	—	—	1	—	2	2	2	—	—	1
B	16	3	9	3	1	8	2	5	—	—	—	1	—
C	7	2	3	—	2	3	2	1	—	—	1	—	—
D	28	10	15	3	—	19	1	4	4	—	—	—	—
E	9	3	—	5	1	3	1	2	—	—	—	—	3
Total	68	21	32	11	4	34	6	14	6	2	1	1	4

TABLE 7

Changes in the T Waves

Distribution of the cases within the various groups as to age (children vs. adults) and treatment series. The percentage in terms of the total number of cases in the series is given in the bottom row.

Group	Number	Adults (279 cases)	Children (2552 cases)	Penicillin cases (1368)	Control cases (539)	Desquamation cases (505)	Other cases (419)
A	8	7	1	1	3	3	1
B	16	7	9	9	6	1	—
C	7	2	5	2	2	1	2
D	28	5	23	15	6	2	5
E	9	1	8	6	1	1	1
Total	68	22	46	33	18	8	9
% of total		7.9%	1.8%	2.4%	3.3%	1.6%	2.1%

In addition to myocarditis a negative T wave may also be the result of pericarditis. In pericarditis, however, the alteration is felt to be ordinarily found in all of the standard leads, and in earlier stages there are elevated S-T segments. Two such cases are observed in this study. One of these (record no. 4821/47) is reported below (page 125). Another patient, a male (record no. 1888/48) had a negative T wave and a slightly elevated S-T segment in all of the extremity leads. In lead CR_4, however, the T wave was highly positive, and there were no clinical signs of pericarditis. However, also discordant alterations of the terminal deflections in different leads may be caused by a pericarditis, especially when it is localized. (See among others Burchell et al. 1939, Goldberger 1947, and Scherf and Boyde 1948.) The possibility of pericarditis has therefore been considered in patients with negative T waves. A number of them have been carefully followed by auscultation although neither the so-called "friction rub" was noted, nor was there evidence of pericarditis when x-ray examination was made.

Group A

In this group are included cases in which T_1 and/or T_2 were negative at any time and subsequently became positive again. This significant change has been found considerably more often in adults than in children. In the adults they were observed 7 times in the 279 patients or 2.5 per cent, while in children they were found in 1 out of 2552 or 0.04 per cent. This is a statistically significant difference. The changes were observed for from 2 weeks to 2 months (see table 6). A one-occasion appearance of such a change was observed only once in the routine electrocardiograms. In the child (record no. 3014/48, page 108) the change was of long duration but the outcome is unknown since she did not appear for the follow-up examination.

Only 2 of the 7 patients who were interviewed following the illness have complained of dyspnea and marked fatigue. A third patient described atypical difficulties. Precordial pain has not been reported. Follow-up examinations from 1 to 4 years following the illness have been made on 6 patients, and in all of them the T waves were entirely normal both during rest and after work. One patient, however, developed extrasystoles during the work electrocardiogram (record no. 3616/47, page 70). The function tests were also normal, but 2 of the women reported that they had not regained their former condition following the illness.

A 39 year old woman (record no. 4821/47) showed an elevation of S-T in leads II and III (figure 32 a) in the first week. Three days later the T waves were generally flattened; and after another 10 days T_2 and T_3 had become deeply negative while T_1 was negative to diphasic (figure 32 b). This series of electrocardiograms is extremely suggestive of pericarditis. Repeated auscultation as well as x-ray were normal, however, although of course it may have been a pericarditis in spite of this. Other than a certain degree of fatigue the patient had no complaints. One week later T_1 was normal but T_2 and T_3 were deeply negative (figure 32 c). This pattern persisted for 3 weeks. Subsequently T_2 and T_3 slowly came up (figure 32 d), but it was only after another 2 months that the electrocardiogram was entirely

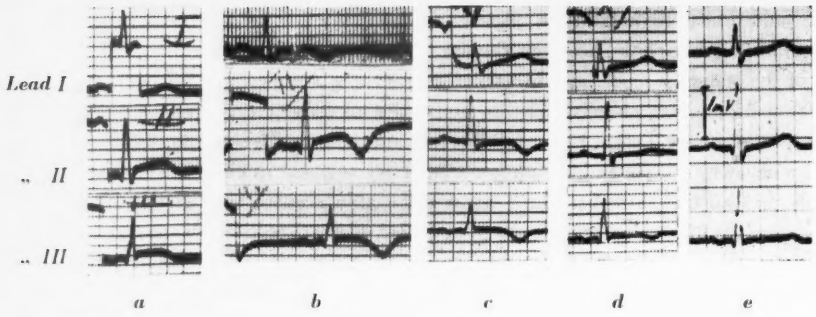


Fig. 32. *Development and regression of a myocarditis as revealed by changes in the T waves.* (Record no. 4821/47.)

a. Recorded during first week of illness. S-T_2 is obliquely elevated, S-T_3 elevated and plateau-shaped. The T waves are positive.

b. The third week of illness: the S-T segments are isoelectric, and the T waves are deeply negative in leads I to III.

c. The fourth week: T_1 is positive, T_2 and T_3 are negative.

d. The eighth week: T_1 is positive, T_2 slightly positive and T_3 slightly negative.

e. Four months after the illness all T waves are positive.

normal with definitely positive T waves in leads II and III (figure 32 e). After discharge the patient was advised to take a long rest and avoid exertion. She was subsequently asymptomatic. A function test on the bicycle ergometer was carried out 5 months after the electrocardiogram had become normal and 9 months from the onset of illness. At this time she was able to manage light loads but not the moderate one of 600 Kg.M./min. Even after work, however, the electrocardiogram was normal. The patient then began slowly to increase her physical activities, and at the time of the follow-up examination 2½ years later she was following a normal program but felt that she still became short of breath more easily than previously and that she had not fully regained her original condition. However, she was now able to manage the moderate load, and both the standard and precordial leads at rest and after work were normal. It would thus seem that restitution had taken place in this case.

Another of the patients with negative T waves was followed with frequent electrocardiographic examinations, and in this case it was possible to follow the lowering and subsequent elevation of the T waves. This was a 21 year old student nurse (record no. 407/48) who was assigned to the control series without penicillin therapy. However, due to a complicating otitis, she began to receive penicillin after the fourth hospital day. Her electrocardiographic alterations first appeared on the tenth day of illness (5/2), and figure 33 shows her electrocardiographic series up to the time when the electrocardiogram was found to be practically normal on 12/2. The alteration was present in only one of the routine electrocardiograms, and in the table it is thus reported as "once" under duration. The patient was kept quiet for 3½ months after normalization and was subsequently asymptomatic when she began work.

In conclusion it may be said that even if this considerable change, which was found most frequently in adults, was of relatively long duration recovery oc-

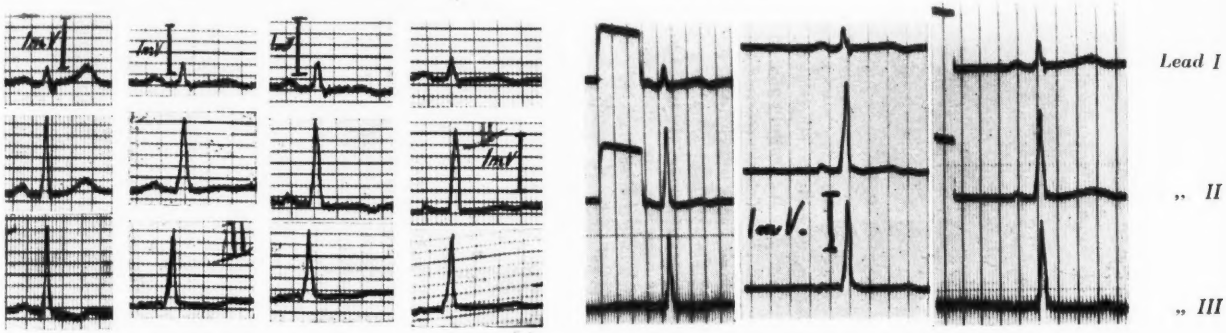


Fig. 33. *Transient inversion of the T wave, observed in daily recordings.* (Record no. 407/48, leads I—III.)

a. On the 9th day of illness (4/2) at a frequency of 95, T_1 and T_2 are positive.

b. 5/2. Frequency 80. T_1 is flattened, T_2 is isoelectric.

c. 6/2. Frequency 75. T_1 is diphasic to negative and T_2 negative.

d. 7/2. Frequency 70. T_1 is diphasic to negative and T_2 slightly positive with a double apex.

e. 10/2. Frequency 55. T_1 is diphasic and T_2 slightly positive with a double apex.

f. 11/2. Frequency 55. T_1 is diphasic and T_2 positive with a double apex.

g. 12/2. T_1 is positive; T_2 is also positive although still somewhat low.

curred. It is noteworthy that 3 of these 8 patients were controls and 3 belonged to the desquamating series. The only case in the penicillin-series belonged to series P_5 and received treatment beginning on the 5th day in the hospital.

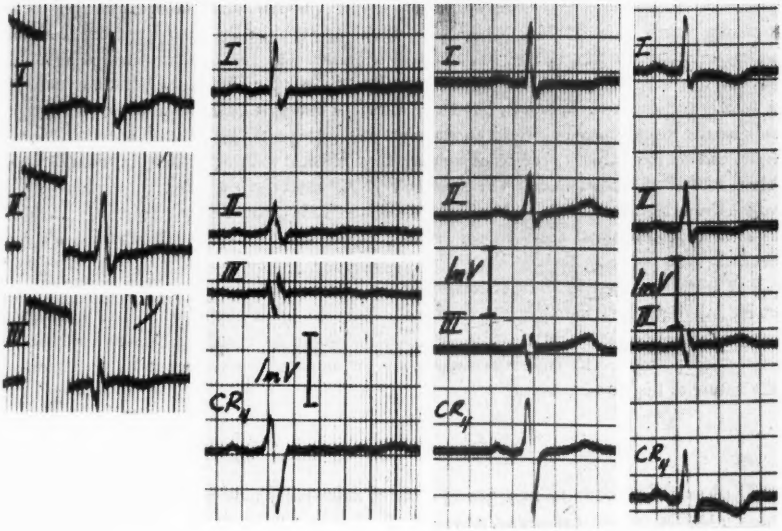
Group B

An isoelectric T_1 and/or T_2 have been observed in 16 patients — 7 adults and 9 children. Six of the patients had transitory symptoms such as tingling pains over the precordium, shortness of breath, and marked fatigue. The course of the heart disease appeared to be shorter than in the preceding group and more than half of the cases had demonstrable changes in only one routine electrocardiogram (see table 6). The others continued for from 1 to 8 weeks, with one exception, a 46 year old woman (record no. 921/47) who developed a persistent change.

In her youth this patient was treated for glandular tuberculosis, and a few years previous to the scarlet fever she had received thyroid treatment for a moderate hypothyroidism. She had never previously had any heart complaints and was assigned to the control series. In the third week of illness she developed an erysipelas of the face for which she received a sulfa preparation. She then developed an urticaria which was probably due to sulfa hypersensitivity. The patient's first electrocardiogram on the sixth day of illness (figure 34 a) showed a positive T_1 and a practically isoelectric T_2. In the next electrocardiogram (figure 34 b), in the third week of illness, all the T waves were practically isoelectric. The patient was discharged a short time thereafter to a convalescent home. A few days later she suffered a severe attack of angina pectoris and was admitted to a local hospital where she was treated for 6 months on suspicion of cardiac infarction. Definite evidence of an infarction, however, was not found either at this time or later at a follow-up examination, but she had an obvious coronary insufficiency and a pathological work electrocardiogram with inversion of the T waves (figures 34 c and d). Ever since her scarlet fever she has been troubled by effort angina. Unfortunately the difficulty increased by the thyroid medication which the patient was so dependent upon since she otherwise was psychically sluggish and increased in weight. It was also clear that the electrocardiographic changes were more pronounced during thyroid medication, and for this reason her treatment was difficult to regulate.

This woman thus developed persistent symptoms of coronary insufficiency during the scarlet fever period. It must of course be questioned whether there was not a coronary sclerosis even earlier despite the absence of symptoms. The first electrocardiogram with flattening of T_2 was not entirely normal but this may have been related to the tachycardia. The change in lead I, however, appeared during the scarlet fever and probably also as a result of it. The patient had a severe case of scarlatina which in addition was complicated by an erysipelas. Her anti-streptolysin titer reached the high value of 1:6400. She also demonstrated an allergic reaction which was probably a factor in the development of intimal damage in the vessels.

As Rich and Gregory (1943) have shown experimentally, serum sickness may produce "fibrotic intimal lesions" in the coronary as well as in the periferal arteries of rabbits. These resemble those seen in humans with rheumatic fever and periarteritis nodosa, and angina pectoris with subsequent development of an infarct two months later has been described in a 32 year old man subsequent to serum sickness (Czickeli 1950). As shown by de Brux (1948) and Plotz (1948) lesions in the coronary vessels are not too uncommon in infectious diseases even in younger subjects. According to de Brux the cicatrization stage of rheumatic coronary disease resembles arteriosclerosis. For this reason it must be asked whether vascular



a b c d

Fig. 34. *Persistent alteration of the T wave and pathological work electrocardiogram in a case complicated by hypothyroidism.* (Record no. 921/47.)

a. Leads I—III on the 6th day of illness. The frequency is 100. T_1 is positive, T_2 slightly positive, T_3 negative.

b. Leads I—III and CR_4 in the 3rd weed. The frequency is 70 and the T waves are isoelectric.

c. Leads I—III and CR_4 in rest 1 year after the scarlet fever. The frequency is 55. T_1 is diphasic, T_2, T_3, and T_4 are positive.

d. The leads taken at the same time as figure c. immediately after work. T_1 and T_4 are negative; T_2 is diphasic and T_3 positive.

damage as a result of infectious processes may not undergo atheromatous alterations. It seems possible that there was an infectious or perhaps allergic coronaritis in this case. The fact that the patient probably had a hypercholesterinemia as a result of hypothyroidism possibly contributed to the development of atheromatous permanent coronary damage as well as to the disease picture as a whole. The scarlet fever, however, would seem to have been the immediate factor.

Another patient in this group is worth mentioning. This was the case of a 9 year old girl (record no. 1981/47) who displayed an isoelectric T_2 on the thirteenth day of illness. This subsequently became positive and would seem to indicate an acute myocarditis. At the follow-up examination four years later it was learned that the patient had always seemed to be in poor physical condition and to have difficulty in keeping up with her comrades although this was not more noticeable following the illness than previously. She had a consistant tachycardia of about 120 beats per minute, and a harsh systolic cardiac murmur could be clearly heard in the fourth left interspace bordering the sternum. The blood

pressure was 90/70. The electrocardiogram in rest showed sinus tachycardia and wide auricular waves of about 0.11 to 0.12 seconds in duration. In the limb leads the T waves were isoelectric; and in IV R, CR_5, and CR_7 diphasic. The function test showed that she patient was capable of only 300 Kg.M./min., but the work electrocardiogram was normal. A phonocardiogram taken shortly after the work test revealed summation gallop. The blood volume was determined by Sjöstrand's carbon monoxide method and was reported, "Large heart volume in relation to the blood volume. Low physical working capacity in relation to the blood volume and heart volume (T. Sjöstrand)". The report of the heart x-ray read, "Heart volume measured to be 450 cc./M.^{2} of body surface. Enlargement of the heart of such form as to indicate congenital heart disease (Edling)". The basal metabolic rate was minus 2 per cent.

The history as well as the electrocardiographic changes, which were of a different type at the follow-up examination from those seen during the illness, seem to indicate a probably congenital heart disease rather than a residual following an acute myocarditis at the time of the scarlet fever. The patient is supposed to be admitted for further study to determine the nature of the cardiac disease.

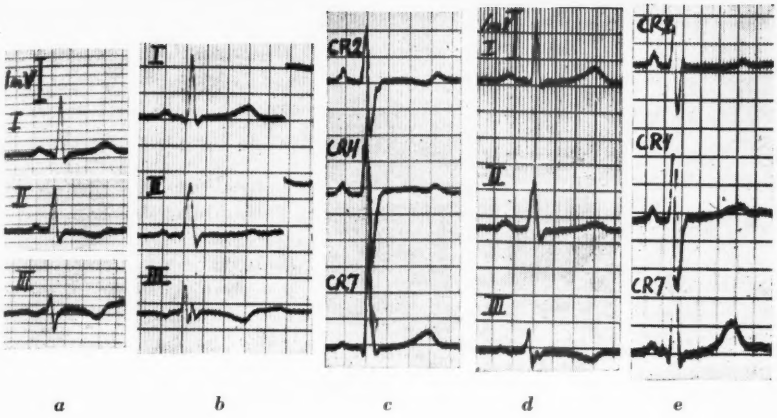
Group C

Diphasic T waves, usually going from a negative phase to a positive one, have been observed in 7 patients of whom 2 were adults. As in the preceding group the changes have usually been of short duration. Three of the patients had subjective complaints. One young man reported anginal pains for a long time after normalization had taken place although both the function test and work electrocardiogram were normal. However, this subject was of neurasthenic type, and this may have played a part in the persistence of the symptom. In the case of one 6 year old girl a very marked fatigue and lack of endurance were observed for a short while following the illness.

The third patient with subjective complaints was a 9 year old girl (record no. 4682/47) who belonged to series C_2. She displayed the most severe changes in this group. At the time of onset she had a synovitis and displayed a diphasic to negative T_2 and depressed $S\text{-}T_2$ for 5 months (figure 35 a). Subsequently T_2 became isoelectric to suggestively positive and after one year was slightly positive (figure 35 b). In the precordial leads the T waves in CR_2 and CR_4 were diphasic and lower in CR_4 than in CR_2 (figure 35 c). At a follow-up examination 2½ years later the T waves in leads II, CR_2 and CR_4 were clearly positive (figure 35 d and e) and higher in CR_4 than in CR_2. The precordial lead T waves of children normally become positive from left to right during the course of development, but the above finding with T waves lower in CR_4 than in CR_2 and the subsequent reversal of this relationship would seem more likely to be evidence of the regression of a myocardial lesion. One year after the illness the patient is supposed to have had a "heart attack" according to the personnel of a summer camp where she was staying, but it was impossible to get a description of this. At the present time the patient takes an active part in play and gymnastics like other children. Thus it would appear that this girl had a myocarditis with a change lasting for a year or more with subsequent gradual normalization.

Group D

A definite flattening of T_1 or T_2 has been found in 28 patients — 5 adults and 23 children. In 10 of these cases there has been an associated alteration in the



a *b* *c* *d* *e*

Fig. 35. *Protracted changes in the T wave.* (Record no. 4682/47.)

a. Leads I—III recorded during the scarlet fever. T_1 is positive, T_2 diphasic, T_3 negative.

b. Leads I—III 1 year after the disease. T_1 is positive, T_2 slightly positive, T_3 negative.

c. Leads CR_2, CR_4 and CR_7 recorded at the same time as b. T is diphasic in positions 2 and 4 but lower in 4. T_7 is positive.

d. Leads I—III two and a half years after the scarlet fever. T_1 and T_2 are distinctly positive, T_3 is negative.

e. Leads CR_2, CR_4 and CR_7 recorded at the same time as d. The T waves are positive in CR_7 and CR_4 and positive to diphasic in CR_2. The amplitude is higher in point 4 than in point 2.

form of the T waves with two peaks or a plateau contour. The diagnosis of myocarditis in 8 cases has been substantiated by depression of the S-T segment, and in a few cases there were other changes such as premature beats, nodal rhythm and pre-excitation.

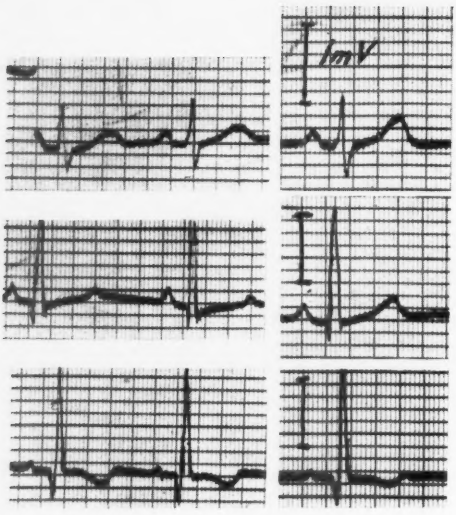
These small changes in the T wave have in two-thirds of the patients regressed within one week. Complete normalization has occurred in all cases. Only a few of them have had transitory cardiac complaints, consisting of shortness of breath or palpitations during the earlier convalescence.

The case of a 5 year old boy (record no. 1867/49) is offered as an example of this group of changes. On the fifth day of illness T_2 was low and abnormally shaped (figure 36 a). Figure 36 b shows one of his later electrocardiograms following normalization with a highly positive T_2.

Group E

Equivocal Lowerings of the T wave in Association with Heart Complaints

Obviously there is an indefinite borderline between lowerings of the T wave which might be regarded as indicative of myocardial disease and those to which no importance can be ascribed. Lowerings that could not be dismissed as insigni-



a *b*

Fig. 36. *Lowering of T_2 in association with change of form.* (Record no. 1867/49, leads I—III.)

a. Recording from fifth day of illness showing sinus arrhythmia at a frequency of approximately 90. T_2 is positive but of abnormal form, and there is a marked positive after-potential.

b. All of the patient's other electrocardiograms were of this type with the same frequency but highly positive T_2. (There is some difference in the calibration of the apparatus in the three leads.)

ficant but which on the contrary did not seem to justify a diagnosis of myocarditis by themselves have been observed in 18 cases. In such cases it is obviously important to consider the clinical picture just as in those cases where the electrocardiographic changes are of a sympatheticotonic type. In 5 of these patients the associated subjective complaints referable to the heart in conjunction with the electrocardiographic findings have been felt to justify a diagnosis of myocarditis. Two of these (record nos. 3857/48 and 88/49) have already been reported with the suspected pathological work electrocardiograms (page 121), while in another case there was pre-excitation (page 147, figure 42). The two remaining cases (record nos. 3500/46 and 1405/49) displayed slight flattening of T_1 and T_2 and complained of shortness of breath following the scarlet fever. These 5 cases are reported in a separate group, group E. This consists of patients with equivocal flattening of the T wave whose subjective complaints indicated the presence of heart disease. The 3 previously mentioned patients with T wave depressions of the sympatheticotonic type, and one patient with an alteration possibly due to variations of heart position have also been concluded in this group, which thus includes 9 cases, 1 adult and 8 children.

Summary

A survey of the T wave alterations in the various series and ages as well as their onset and duration is given in tables 6 and 7. These were the most common finding in this study. In general it may be said that even more pronounced changes with negative T waves display a definite tendency to regress. They

persisted in only one case, that of an elderly woman. Even minor changes with flattening of the T wave may have a clinical significance since they are not uncommonly associated with cardiac symptoms. A more detailed analysis of the frequency and degree of the changes in adults and children as well as in various treatment series is given in the latter part of the book (page 162, figure 45 and page 171).

Variations in the T Wave in Lead III

The T wave in lead III is regarded as being markedly variable even under normal conditions. However, there are probably myocardial lesions which will be manifested chiefly or solely in lead III when only the three standard extremity leads are used (Buchem and Daniels). In addition there are of course a number of significant variations in T_3 which occur simultaneously with changes in T_2. Marked changes in T_3, as for example from positive to negative, should therefore be taken into consideration. However, a diagnosis of myocardial damage can be made only after supplementary studies of the variations in T_3 consequent to changes of frequency and position in the patient in question.

Large alterations in T_3 of the type which might be related to changes of position have been reported earlier (page 116, figure 25). In contrast to these patients is a 10 year old boy (record no. 3862/48), who had a negative T_3 and slight depression of the S-T segment in his first recording, while in subsequent electrocardiograms recorded at the same pulse frequency T_3 was positive and the S-T segment isoelectric. At the follow-up examination three years later T_3 was positive in all positions. Some time after the scarlet fever, but not immediately following, he had complained of pain in the region of the heart. This alteration is highly suspicious, but due to the long interval to the follow-up examination the author does not feel justified in classifying this as other than an equivocal change. A similar questionable alteration was also observed in the case of a 7 year old girl (record no. 3396/47).

As pointed out in the discussion on sympatheticotonic electrocardiographic alterations on page 111, an inversion of T_3 of such a nature that it may have been related to a coincident increase in frequency has been seen in 6 patients. On the other hand, subjective heart difficulties were noted by no less than 3 of these patients, thus seeming to indicate a myocarditis.

In 2 patients a previously observed inversion of T_3 in association with a tachycardia could not be reproduced at the follow-up examination. One of these, an 8 year old girl (record no. 4645/48), was short of breath on exertion and fretful following scarlatina, and it would therefore seem likely that the heart was involved. The other patient (record no. 3826/48) had, however, more indefinite complaints, and the diagnosis of myocarditis is regarded as questionable in his case.

Changes in T_3 of an apparently significant type were observed in two other patients (record nos. 3337/47 and 1302/49) who had in addition depression of the S-T segment which in itself justified a diagnosis of myocarditis.

Thus there were a total of 4 patients with a diagnosis of probable myocarditis in whom the predominant alteration was seen in T_3.

The Q-T Interval

In scarlet fever prolongation of the Q-T interval seldom indicates a myocarditis that is not manifested also by other changes in the electrocardiogram (Spang and Welsch 1947, Bengtsson et al. 1951). In a smaller number of patients, about 200 cases, the Q-T times were studied but no additional information was gained. Besides, when the Q-T time is of diagnostic importance simultaneous recording of the phonocardiogram is often necessary to analyze the disturbance. For these reasons the Q-T times have not been systematically investigated in this material.

CHAPTER 11

Changes in the S-T Segment

Introduction

Alterations in the S-T segment are often associated with changes in the T waves. They may also occur independently as evidence of myocardial damage.

Normal Variations

a) Deviations from the Isoelectric Line

It is not uncommon for the S-T segment to differ in its level from that of the reference level even in perfectly healthy individuals. The segment between the end of the P wave and the beginning of the QRS complex is chosen as reference level because it is most useful even at higher frequencies.

Adults: Elevation often occurs to a noteworthy degree in cases of increased vagotonia. Elevations of as much as 2 mm., in particular in lead II, are reported by both Graybiel et al. and Stewart and Manning in their large series of normal adults. *Depressions*, however, have been observed more rarely and in less marked degree, and only 5 people altogether had S-T depressions of more than 1 mm. out of these 1,500. This would seem to be the general experience, and in agreement with this it has often been reported that the normal range of variation is greater in the upward direction than in the downward. Rasmussen (1946) as well as Scherf and Boyde (1945) give an elevation of 0.20 millivolts, and Rasmussen a depression of 0.10 millivolts, as the normal limits for the extremity leads. The same limits for both elevation and depression are given by the Nomenclature for 1945 (0.10 millivolts) and by Burch and Winsor (1949) — (0.15 as pathological, 0.10—0.15 as suspicious). However, since different authors measure the depression at different parts of the S-T segment the values are not always comparable.

Children: In children other than infants normally only minor changes in the S-T segment occur. Elevations or depressions of more than 0.10 millivolts are generally regarded as abnormal (Hafkesbring 1937, Mannheimer 1940, Nadrai 1941, Seham and Moss 1942, and Harris and Dwan 1948).

b) Changes in Contour

Aside from the magnitude of the deviation from the reference level the contour of the S-T segment has a definite and often decisive diagnostic significance.

Parkinson and Bedford (1928) described three types of abnormal, elevated S-T segments — plateau-shaped, dome-shaped, and obliquely elevated. The elevated S-T segment of normal cases has a downwardly directed convexity which may, however, be present even with myocardial damage. As Goldberger (1950) showed, some of these abnormal cases have a special sort of convexity which he called "crescent" S-T. In this type the lowest point on the S-T segment is equidistant from the take-off and the top of the T wave. In other cases the lowest point of the S-T segment is closer to the take-off, and the change can then be differentiated from the normal variation only by the degree of elevation. I suggest that this also normally occurring type of S-T elevation be called "uphill" S-T.

With regard to depression of the S-T segment, there is one type which should be interpreted as abnormal only with the greatest caution. In this the actual S-T "take-off" or junction is depressed, but a rapid and immediate rise follows. This type is usually seen in the presence of a sympatheticotonia or in normal subjects following marked exertion or hypoxemia. (See Scherf 1947 and Sjöstrand 1951.) An analysis of this type of change (Sjöstrand 1950) has shown that they are probably "relative", the result of an elevation of the reference level — the P-Q segment — by a positive after-potential. A negative auricular T wave which becomes more negative following work has also been suggested as a cause of such depression of the S-T segment (Scherf 1947).

Bases of Interpretation

Depressions of the S-T segment of 0.1 millivolts or more have been considered suspicious. Considerably greater elevations (up to 0.2 millivolts) have been accepted if the S-T segment did not have an abnormal form. The frequency and autonomic state as well as the patient's series of electrocardiograms have been taken into consideration in interpreting these aberrations. To establish fixed normal limits is impossible except under basal conditions and in autonomic equilibrium.

The Author's Cases

I. Elevation of the S-T Segment

Elevations in the S-T segment have been observed a number of times. With a few exceptions, which are mentioned below, they have all been of the rounded downward convex type — "uphill" S-T — which is usually seen in healthy subjects. They seem to have been entirely independent of the illness and often have remained unchanged. In many cases they have been accompanied by high, positive T waves such as are usually seen in vagotonic subjects.

There was a pathological elevation of the S-T segment in the previously described case with suspected pericarditis (page 125, record no. 4821/47). When the change first appeared in lead II there was an "obliquely elevated" S-T

(figure 32 a). In lead III the S-T segment was of a "plateau" form. Although these elevations amounted to only 0.1 millivolts they are probably significant. In another 2 cases elevations of the S-T segment of the "dome-shaped" or "plateau" form have been observed in association with negative T waves, and minor elevations together with flattening of the T waves in two patients.

II. Depressions of the S-T Segment

a) Changes Associated with Sympatheticotonia and Tachycardia

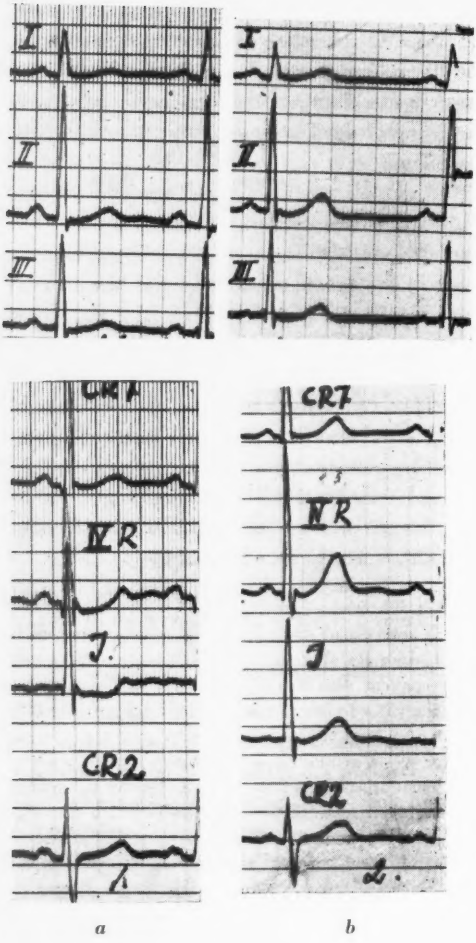
Aside from cases of myocardial damage, depression of the S-T segment as well as of the T wave may be seen in the functional state of the heart which accompanies tachycardia and sympatheticotonia resulting either from the illness or constitutional factors. This depression of the S-T segment is often of the "low junction" type. As in the case of T wave changes (see page 105) the amyl nitrite and ergotamine tests and prolonged observation have been used for purposes of making a differential diagnosis, and serial electrocardiograms have been compared with respect to frequency and sympatheticotonic effect.

Subsequent to the above-mentioned tests the possibility of changes due to autonomic influences has been confirmed in a number of cases with obvious S-T depressions both with and without associated flattening of the T waves.

Thus an 8 year old girl (record no. 1178/49), who is mentioned earlier in the section on T wave alterations (page 108), also had a lowering of the S-T segment which amounted to 3 mm. in lead IV (figure 20 a). However, since the alterations were constant during a long period of observation, during which the patient was free of symptoms, and disappeared under the effect of ergotamine (figure 20 b), it was felt that a constitutional sympatheticotonia was the cause.

Another 8 year old girl (record no. 305/49) also illustrated the type of change which is regarded as being a result of a marked constitutional vegetative lability. During an observation period of one and a half years she had depressions of the S-T segment which increased following work with a tachycardia. This depression remained even after ergotamine. Immediately following her illness she was "terribly tired", but at the follow-up examination one and a half years later she was as alert as usual and jumped rope vigourously every day. In the electrocardiogram which was recorded at rest the S-T segment was depressed 0.5 mm. in leads II and III. In leads IVR and J the depression amounted to 2 mm. (figure 37 a). A new recording was made three days later. At this time she came directly from a strenuous gymnastic lesson, but in spite of this the frequency in rest was lower, and the S-T segments were very nearly isoelectric (figure 37 b). She managed a function test of 300 Kg.M./min. for six minutes without difficulty. Following this the S-T segment was again depressed between 1 and 1.5 mm. both immediately and 4 minutes afterwards. When pressure was applied to the carotid bodies and the eyeballs during rest a definite elevation of the S-T segment above the isoelectric line occurred. Upon inhalation of amyl nitrite vapors a marked depression resulted. There was thus a marked sensitivity to various vegetative factors. Of course it is possible that an early myocarditis with persistent myocardial damage was present, but since it seems more likely that the entire phenomenon resulted from marked constitutional lability the diagnosis of myocarditis has been regarded as uncertain.

An analogous situation was present in still another girl of 10 years (record no. 4771/48). This patient displayed minor depressions of the S-T segments at rest which increased following work even when ergotamine was administered. At the follow-up examination 18 months



a *b*

Fig. 37. *Persistent depression of the S-T segment markedly influenced by vegetative factors.* (Record no. 305/49, limbs leads and precordial leads CR_7, IVR, J, and CR_2.)

a. Recording one year after the scarlet fever showing slight depression of the S-T segment in leads II and III, marked depression in IVR and J, and a low T wave in lead I with a frequency of 95—100. Note marked after-potential!

b. Recording three days later with a frequency of 75 showing practically isoelectric S-T segments.

later the rest electrocardiogram was normal, but after the work test the S-T segment was still depressed between 1 and 1.5 mm. The patient was, however, completely free of symptoms. On the basis of the persistent tendency toward a depression of the S-T segment it is felt, despite the ergotamine test, that this is most probably a case of extreme vegetative lability.

When changes in the electrocardiogram appear to be caused by vegetative factors it is important to consider the clinical picture since the presence of vegetative influences does not rule out the possibility of a myocarditis. This point has been brought out previously in discussing the flattening of the T waves. The 9 year old girl (record no. 3889/47) mentioned above (page 111) was classified as having a myocarditis because of obvious cardiac symptoms in

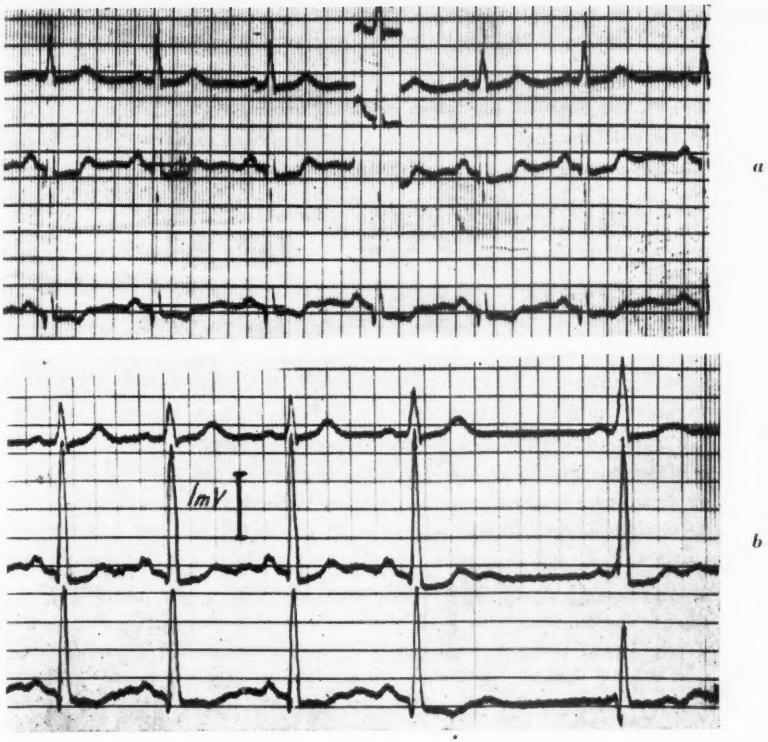


Fig. 38. *Two types of depression of the S-T segment in work electrocardiogram.* (Record no. 4263/48, leads I—III immediately after work.)

a. 21st day of illness. S-T segments in leads II and III are depressed 2 mm. and are plateau-shaped; the frequency is 100—110. This work reaction is probably abnormal.
b. 1 year after the scarlet fever. Depression of the S-T segments of the "low junction" type with a sinus frequency of 120. This work reaction is within normal limits. After a prolonged systolic interval perisinus rhythm and pre-excitation appear.

association with an inverted T_3 and depressed S-T segments. An 8 year old girl (record no. 4179/48) displayed during the illness a suspicious lowering of the S-T segment which, however, appeared again in the follow-up examination although to a less marked degree. In addition, she was found to have a high frequency nodal rhythm in the third week of illness, and this in itself justifies the diagnosis of myocarditis.

b) Equivocal Depressions of the S-T Segment

When it is extremely ticklish to draw the line between normal and pathological variations as in the present instance, one is of course frequently faced with

depressions which make it difficult to take a definite position. The alteration may be on the borderline of normal but may still differ clearly from the patient's other electrocardiograms. Changes may also occur which seem to be only partly explainable on the basis of vegetative factors. Such equivocal S-T depressions have been observed in 8 patients. Three of them have been classified as probable myocarditis cases with heart symptoms, since they complained of dyspnea and palpitation following scarlet fever.

c) Depressions of the S-T Segment Ascribed to Myocarditis

Depressions of the S-T segment of significant magnitude, which did not seem to be due to vegetative factors, and which appeared during the illness and regressed, have been interpreted as evidence of myocarditis. Depressions of the S-T segment were the only evidence of myocardial damage in 4 cases. In another 5 patients there were in addition definite T wave changes and in 2 patients changes in T_3. Four of these 11 patients reported subjective difficulties — 3 shortness of breath, and 1 palpitations. The changes were transitory in all cases.

Further depression resulted from the work test in some cases, but in all patients work reaction also has finally become normal.

A 9 year old girl (record no. 4263/48) displayed a depression of the S-T segment amounting to 1 mm. and a flattening of T in lead II. On the following day the depression had decreased but immediately and 4 minutes after work there was a marked plateau shaped depression of S-T in leads II and III (figure 38 a). This work reaction supports the diagnosis of myocarditis. The girl was tired and short of breath on climbing stairs a short time after the illness. At the follow-up examination 1 year later there was also a lowering of the S-T segment in the work electrocardiogram, but the depression was now of the "low junction" type and seemed to be within normal limits. In addition the patient displayed pre-excitation, which appeared at the end of the curve in figure 38 b.

Minor insignificant depressions of the S-T segment have appeared together with other unequivocal signs of myocarditis in 7 cases and together with equivocal changes in cases with probable myocarditis and heart symptoms 6 times. In the majority of these cases, 12, the other change was an alteration of the T wave.

In conclusion it may be said that abnormal *depression* of the S-T segment as a result of myocarditis has been observed in 11 patients. In another 4 patients with S-T depressions of equivocal or sympatheticotonic nature subjective heart complaints made a myocarditis probable. *Elevations* of the S-T segment were abnormal in type in only 3 cases, who all had inversion of the T waves.

CHAPTER 12

Intraventricular Conduction Disturbances

Normal Subjects

Adults: With regard to the duration of the QRS interval, a value of more than 0.10 seconds has generally been regarded as abnormal (Nomenclature, 1945). In a large group of normal subjects, however, this value is not uncommonly exceeded. For example in Graybiel et al.'s (1944) group of 1000 men, 27 had a QRS interval of 0.11 seconds and 8 one of 0.12 seconds. There was only one with as much as 0.13 seconds. Corresponding values were found by Stewart and Manning (1944) in their group of 500 in which $M+2\sigma$ was 0.10 seconds and $M+3\sigma$, 0.12 seconds. Grewin (1948) gives the upper normal limits as 0.11 seconds (borderline 0.12) for men and 0.10 (borderline 0.11 to 0.12) for women.

Children: In the case of children a value exceeding 0.09 seconds is generally regarded as pathological (see Hafkesbring 1937, Mannheimer 1940, Seham and Moss 1942 among others). Furthermore Mannheimer regards 0.09 seconds as suspicious.

With regard to the shape of the ventricular complex, aberrations have also been observed in normal subjects. Thus for example Graybiel et al. have found one case of a left bundle branch block and Stewart and Manning two subjects with notching in all leads. Graf (1939) found a splintering of the ventricular complex in the same place in a pair of identical twins, and Lepeschkin (1942) claims that individual peculiarities are the cause of the splinterings. One must thus be careful in ascribing them to acquired heart disease.

Earlier Experience in Scarlatina and Rheumatic Diseases

The ventricular complex and its variations, including axis changes, in scarlet fever and rheumatic disease have been the subject of thorough analysis by Wickström (1932), Roelsen (1941), and Crossfield (1946) among others. These authors have found changes in a large proportion of the subjects studied. Prolongation of the QRS time, notching, bundle branch block, and often also changes in the electrical axis have generally been reported along with other changes in the electrocardiogram during the illness (Cohn and Swift 1924, Jacobsson 1946, Orgain et al. 1941, Spang and Welsch 1947, Wendkos and Noll 1944). Usually

the connection between the QRS alterations and the coincident illness has not been analysed. Filberbaum et al. (1946), however, state that the QRS broadening and bundle branch block observed in rheumatic fever remain constant for a long period of observation and for this reason they regard them as probably incidental findings. Similarly Steinmann (1945) emphasizes that notchings and prolongations of the QRS time in a group of scarlatina patients remained constant in contrast to S-T and T changes found in the same patients. For this reason he does not accord them any great importance.

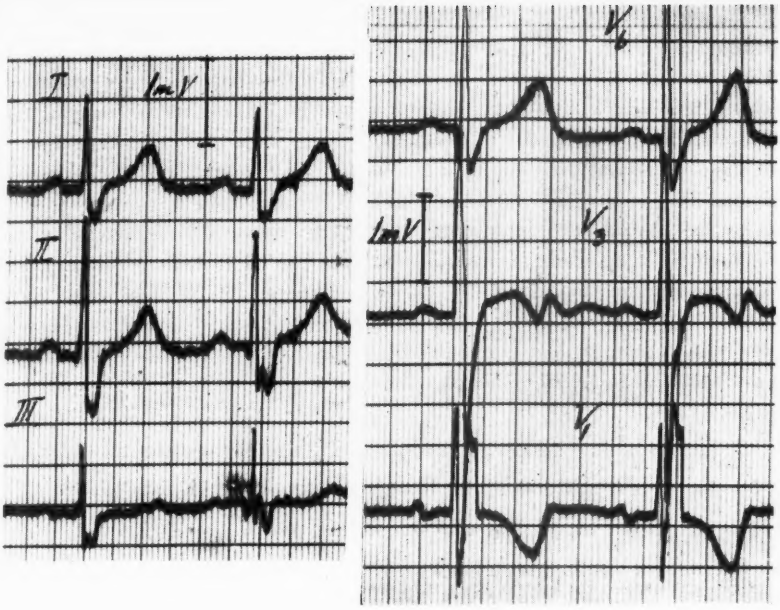
The Author's Standards for Interpretation

For adults the upper normal limit has been set at 0.12 seconds and in children at 0.09 seconds. The usual modern nomenclature of the Wilson school has been followed in the definition and terminology of bundle branch block. Notchings have been regarded as evidence of an intraventricular conduction disturbance only if present in all 3 standard leads. When there was intraventricular conduction disturbance the patient was observed for a long time to see whether the change persisted or not. If possible supplementary precordial leads were recorded in order to localize the lesion more closely. In patients with variations of the axis of the ventricular complex recordings have, so far as possible, been made in various horizontal positions.

The Author's Cases of Intraventricular Conduction Disturbance

A diagnosis of intraventricular conduction disturbance has been made in 29 patients including 3 adults. In 9 patients the conduction disturbance seemed, according to the precordial leads, to be localized in the right ventricle. In 5 of these there were only minor atypical changes. The other 4 cases were atypical right bundle branch block, Wilson type. Two of these (record nos. 1230/49 and 1829/48) were constant in character. The latter was a little boy who was 1½ years old at the onset of the illness. The change in this case has now persisted for 2 years without symptoms. Figure 39 a shows the extremity leads I—III with broad splintered S waves and a QRS interval varying from 0.08—0.10 seconds. Figure 39 b shows leads V_6, V_3, and V_1. A deep broad S_6 and a high broad R'_1 may be seen. A certain variability of the conduction disturbance is present in this case. The two other Wilson blocks were intermittent and are discussed later. Notching and splintering in all 3 leads and/or prolongation of the QRS interval have been observed in the remaining 20 cases of which 2 pairs were siblings with similar changes. Deep wide S waves have often been observed in lead I, but when precordial leads were used they did not help in localizing the disturbance.

It is particularly noteworthy that, of these 29 cases of intraventricular conduction disturbance, 27 have been constant in character. Of these 27 patients,



a b

Fig. 39. *Right bundle-branch block, Wilson type, in a one and a half year old boy.* (Record no. 1829/48.)

a. Leads I—III. QRS varies between 0.08 and 0.10 seconds. The S waves are broadened and splintered.

b. Precordial leads V_6, V_3 and V_1. In lead V_6 the S wave is broadened; in lead V_1 there is a high, broad R' and a negative T wave.

2 have shown other definite signs of myocarditis with changes in the T wave or prolongation of the P-Q time, but these changes have been completely normalized. Fifteen have been observed for a year or more. The patients have not displayed any signs of heart disease and have been free of complaints with two exceptions. One of these was a boy in his early teens who complained of tingling discomfort over the precordium and fatigue. However, this patient displayed a number of neurasthenic characteristics, and the cardiac symptoms probably may be ascribed to this condition. The other case was that of a 38 year old man with a splintered, M-shaped R wave in lead II. The function test after recovery revealed good physical working capacity and a normal work electrocardiogram. Although the recording was unchanged at the time of follow-up examination one year later, he said that he felt himself to be in poorer condition subsequent to the illness than before.

The author is inclined, like Filberbaum et al. and Steinmann, to regard these constant aberrations as incidental findings and unrelated to the scarlet fever. Their etiology may of course be old myocardial lesions the development of which, however, was not indicated by anything in the history. None of the patients, for example, have had diphteria. It would seem likely that the majority of these alterations are congenital or constitutional and often hereditary anomalies. This viewpoint is supported by Graf's study of identical twins as well as by the fact that two pairs of siblings in the present material have had the same change. The situation would seem to be similar to that in certain cases of prolonged auriculoventricular conduction times. The changes could very possibly arise as a result of small anomalies in the conduction system. Theoretically it would not seem that this slight delay of the impulse should produce any functional impairment. This is also in agreement with practical experience in such cases.

Intermittent Bundle Branch Block

Earlier experiences with intermittent bundle branch block

Intermittent bundle branch block seems to be a less common disturbance than constant bundle branch block although a number of cases have been described (Cohn 1913, Cohn and Lewis 1914 [cit. Herrman and Aschmann], Herrman and Aschmann 1930, Jervell 1941, Nichols 1949, Purks 1938, Robinson 1916 [cit. Herrman and Aschmann], Sabattie et al. 1944 and 1945, Segers et al. 1947 and 1948, Slater 1929, Spang and Welsch 1947, Stenström 1923—1927, Willius and Keith 1926, among others). Only one case has been reported in scarlet fever however (Spang and Welsch, 1947).

The appearance of an intermittent bundle branch block suggests a variation in the conduction which, however, may not necessarily be large as shown by Slater, Herrman and Aschmann, as well as Jervell. It may be a question of only a dealy of some hundreths of a second so that the impulse passing over the intact bundle to the one ventricle may reach the other ventricle before it is activated by the impulse coming over its own bundle. In this way a bundle branch configuration may develop. Stenström has also shown how certain intermittent bundle branch blocks closely correspond to first degree A-V block. In other cases, of course, the phenomenon may correspond to second degree A-V block.

The cause of the disturbance is sometimes reported to be organic (arteriosclerosis, rheumatic fever, and infectious diseases) and sometimes functional (hypoxemic or nutritional). Thus Cohn and Lewis (1914) and Robinson (1916) have described transitory blocks without demonstrable organic changes. The majority of cases, however, have been older subjects with cardiovascular diseases, although Nichols reports one case of such disturbance in a soldier with a clinically normal heart.

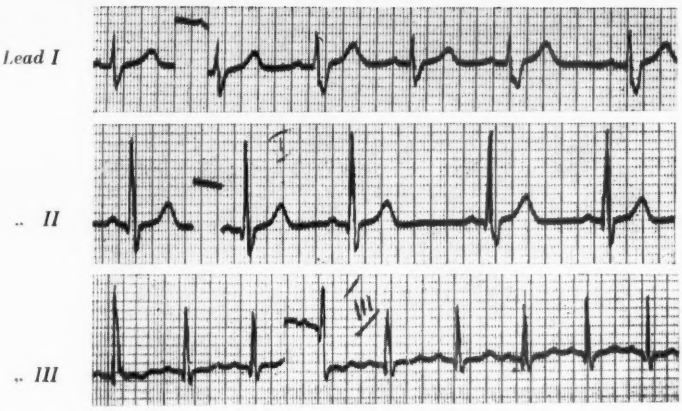


Fig. 40. *Intermittent right bundle branch block.* (Record no. 3925/46.)

The block appears in lead I in the 3rd, 5th, and 6th beats; in lead II in the 4th and in lead III in the 1st beat. Only the last part of the QRS complex is altered.

As Purks and Sabattie have stated, various mechanisms may cause the changes of conduction. This may be a "functional fatigue" when a block appears at high frequency. In others it may be an extreme vagus effect where, in contrast, the block develops at low frequency subsequent to stimulation of the vagus. These different types would seem to have a different significance. In "functional fatigue" the mechanism would seem to be an abnormal type of reaction with increased fatigability. Such cases have been described by Segers and Denolin, and the second case described in this work is one of this type. A vagus produced block on the contrary would seem to indicate an unusual increase in the normal type of reaction with prolongation of the refractory period in one of the bundles. The latter probably could occur both with or without organic damage.

The Author's Cases

Normal ventricular complexes alternating with atypical right bundle branch block (Wilson type) have been observed in two patients. It should be noted that in both cases the scarlet fever was complicated by a virus disease; in one, measles and in the other, varicella. The electrocardiographic changes, however, appeared before the onset of the varicella.

One of these cases was a 3 year old boy (record no. 3925/46) with scarlet fever plus an otitis media and varicella. Electrocardiograms recorded during the hospital stay showed a Wilson block with a QRS time of 0.10—0.11 seconds alternating with more normal ventricular complexes with an 0.08 seconds QRS time. Even the latter had fairly deep and broad S_1 waves (see figure 40). It would seem that a slight delay in conduction in the right bundle branch is normally present in this case. This delay is then increased in certain complexes

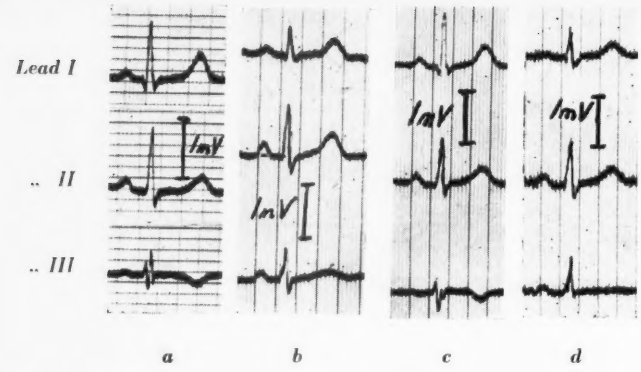


Fig. 41. *Reproduceable variations in the QRS complex.* (Record no. 4440/47.)

Figures a and b show spontaneous variations of QRS observed at different times during the scarlet fever; c and d are recorded at the follow-up examination, c in the supine position, d in the left lateral position. The previously observed variations in QRS reappear.

so that the impulses arrive via the left ventricle more quickly with a resulting bundle branch block configuration. In the figure it may be seen that only the latter portion of the QRS complex (after 0.06 seconds from the beginning of Q) is altered. The disturbance therefore probably involves only the distal portion of the right branch. The block was observed up to 6 months following the illness although the patient did not display any other signs of heart disease. At follow-up examinations 2½ and 3 years later the extremity and the precordial leads both at rest and after work were normal. The patient was able to manage a load of 300 Kg.M./min. in the function test, thus indicating a normal functional capacity for that age.

The other case was that of a 2 year old boy (record no. 2695/48) who was admitted with measles but was also found to be in the desquamating stage of scarlet fever. During a period of 5 months observation he displayed the same features — an atypical bundle branch block, Wilson type, with a QRS time of 0.10 seconds alternating with normal ventricular complexes with a QRS of 0.06 seconds. Again it is only the latter portion of the ventricular complex (after 0.05 seconds from the beginning of Q) which is changed in the blocked beats. Thus the disturbance probably occurs in the distal portion. Accordingly the Q and R waves are unchanged, while the S waves are markedly increased in breadth. Long recordings made under the influence of amyl nitrite show how at higher frequencies the pathological complexes predominate while at lower ones the normal QRS are dominant. It would thus seem that an impairment of the recovery process at higher frequency brings out the block. During a stay in the hospital 2 years later the routine electrocardiogram revealed only normal ventricular complexes.

With regard to the classification of these two cases, there is a time relationship between the scarlet fever and the electrocardiographic phenomenon in both cases with subsequent complete normalization. The second case would appear to be abnormal also on a functional fatigue basis. Both cases have been interpreted as myocarditis.

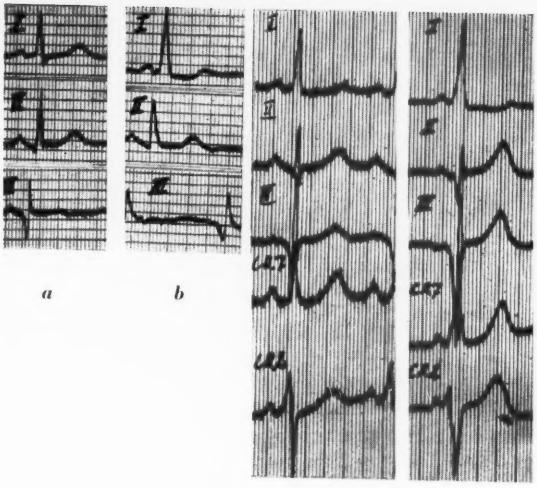


Fig. 42. *Equivocal changes of the S-T segment and the T wave in a case of pre-excitation.* (Record no. 1123/48.)

Figures a. and b. show leads I—III recorded on different occasions during the scarlet fever. In figure a. S-T and T are normal. In b. there is a depression of S-T and T in lead I in association with an oblique rise of the P-Q segment, a possible indication of pre-excitation. Figures c. and d. show two sections of a recording of leads I—III, CR_7 and CR_2 during inhalation of amyl nitrite. In c. the atrioventricular conduction is normal, and in d. pre-excitation appears with a lowering of S-T and T as in figure b.

Variations in the Axis

In association with changes in the T wave spontaneous changes in the ventricular complex with constant QRS time have been discussed (pages 26, 33 and 112). In some cases they could be observed in one and the same reading, indicating a physiological variation. (Figure 22.) In many cases they were seemingly independent of the pathological changes of the T waves in the same patient (figure 26). All cases of axis variations or changes in amplitude investigated in different positions have been reproduceable. It seems therefore possible that they are physiologically normal variations as in the following case.

Figures 41 a and b show two electrocardiograms from a 9 year old girl (record no. 4440/47) which were recorded during the illness and convalescence. These show varying QRS complexes, particularly in lead III. Figure 41 c was taken in the supine position and 41 d in the left-side-down position 2 years after the illness. These duplicate the previously observed variations.

A complete study of the variations of the ventricular complex could be carried out only by the routine use of unipolar extremity and precordial leads. It has not been possible to carry out these basic studies on this large material. In the standard extremity leads no variations of the axis have been observed which could be regarded as definitely indicative of a scarlatina myocarditis.

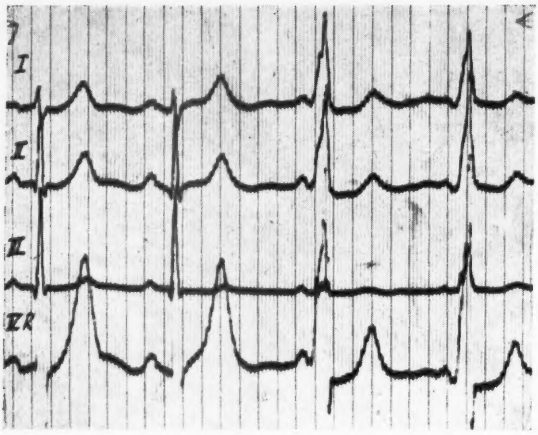


Fig. 43. *Transition from normal atrioventricular conduction to pre-excitation in a 4 year old girl.* (Record no. 616/48.)

Leads I—III and IVR during inhalation of amyl nitrite. The first two complexes are normal and are followed by two beats showing pre-excitation.

Pre-Excitation

In this chapter on intraventricular conduction disturbance it would also seem appropriate to mention some cases of WPW syndrom or pre-excitation (Öhnell, 1944) which have been observed.

In addition to a shortened P-Q time these cases display a ventricular complex of abnormal form with signs of an intraventricular conduction disturbance. This type of change has generally been observed in subjects with apparently normal hearts, and as a rule it has been regarded as constitutional in nature although myocardial disease may sometimes have been present.

Out of the entire material, 7 patients have been observed with electrocardiographic alterations interpreted as pre-excitation. In 5 of these the pre-excitation seemed to be a constant phenomenon which was present in all of the rest electrocardiograms although minor variations of the so-called "concertina effect" type (Öhnell) were observed. One of the patients, a 1 year old girl (record no. 3626/47), moved to an unknown address following her illness, but the other 4 have been observed for a period of 3 years. At the follow-up examinations, at which time recordings were made during deep breathing, inhalation of amyl nitrite, and in the standing position, as well as at rest, a transient normal ventricular complex with an ordinary P-Q time was observed in 2 of the patients.

In the case of a 6 year old girl (record no. 1123/48) the S-T segments and T waves were sometimes seemingly normal (figure 42 a) but in most of the recordings there was a depression of S-T and T particularly in lead I (figure 42 b). The P-Q time and the QRS complex

were within normal limits. However, the P-Q segment in lead I was obliquely rising, suggesting a pre-excitation. There had been a question as to whether these S-T and T changes during the illness might have been the result of a myocarditis. At the time of the follow-up examination it was established that pre-excitation was present and that the observed changes of S-T and T could be reproduced to almost the same degree by amyl nitrite (figures 42 c and d). It is therefore felt that the patients' series of recordings do not provide a definite basis for a diagnosis of myocarditis. At the same time she was reported to have been tired and dyspneic following the illness, and the case has therefore been listed under "equivocal changes with heart symptoms".

A 4 year old girl (record no. 616/48) had no symptoms. Figure 43 shows her electrocardiograms with variations between normal conduction and pre-excitation.

In 2 patients it was impossible to bring out normal conduction, and pre-excitation could be seen in all the complexes even with sympathetic stimulation. One of these was a 10 year old girl (record no. 3233/47) who had experienced no symptoms, while the other was a 24 year old woman (record no. 2107/47). The latter experienced some episodes of tachycardia following the illness and believes that she becomes short of breath more easily than others. She has three children, and the one pregnancy which occurred after the illness followed a normal course. In her first recordings, at which time she had a fever and sinus tachycardia, T_1 and T_2 were deeply negative, while in subsequent recordings they were diphasic to positive. The inversion of T_1 and T_2 would seem to be associated with the tachycardia since it reappeared at the time of the follow-up examination in response to amyl nitrite. The pre-excitation was in all likelihood unrelated to the scarlet fever.

Two of the patients displayed only transitory pre-excitation. One of these, a 12 year old boy, is particularly interesting. Five recordings were made during the course of the illness, the first one 3 weeks after the onset (figure 44 a). In this first one there was a short P-Q time, approximately 0.12 seconds, and a widened QRS complex, 0.10 seconds. Thus the time from the beginning of the P wave to the S-T junction (the "P-J time") was 0.22 seconds. In leads II and III the R wave rose obliquely as in pre-excitation. In lead I the pre-excitation was manifested by an obliquely rising P-Q segment. The frequency was between 85 and 90, and there seemed to be a perisinus rhythm. The T wave in lead I was flattened, probably as a result of the pre-excitation. Three weeks later there was a sinus rhythm in leads I and II, and perisinus rhythm in lead III with a normal P-Q interval (0.14 seconds), while there was a further widening of the ventricular complex (0.12 seconds) with splintering (figure 44 b). The "P-J time" was thus 0.26 seconds. His third electrocardiogram was of appreciably the same appearance as the first. In the fourth and fifth recordings (figure 44 c) the appearance of a Q wave in lead I would seem to indicate a more normal conduction of the impulse. The R wave in leads II and III, however, was oblique in its rise and pre-excitation seemed to be present. In addition the T wave in these leads was diphasic, which, in comparison with figure 44 a, was probably not the result of the altered intraventricular conduction.

At the time of the follow-up examination 5 years later long recordings were made during deep breathing, pressure on the eyeballs, and inhalation of amyl nitrite; but pre-excitation did not appear. (See figure 44 d.) The P-Q time was 0.16 seconds and the QRS complex 0.09 seconds (P-J time thus 0.25 seconds). A Q wave was present in leads I and II. A sinus rhythm was present in rest, but with deep breathing (figure 44 d) the alternating sinus-perisinus rhythm appeared in the usual manner. The precordial leads were normal. The patient experienced no heart difficulties either during or following his stay in the hospital. He did not take part in gymnastics for a time following the illness. When he began again he felt as usual, and at the time of the follow-up examination he was in excellent condition. There were no abnormal auscultatory findings.

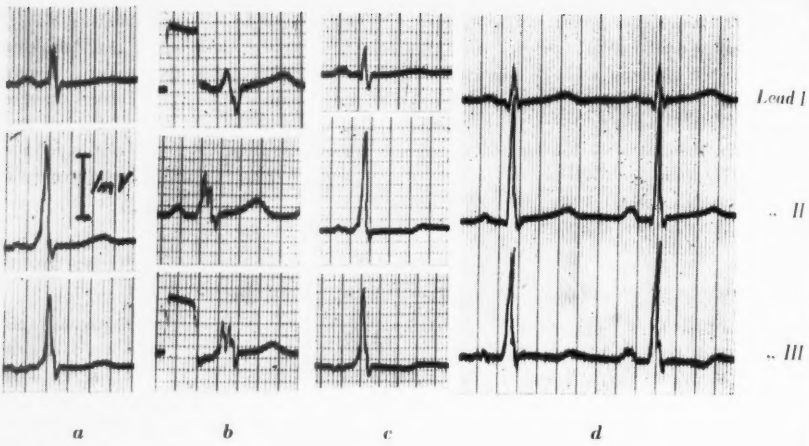


Fig. 44. *Pre-excitation with additional changes in the T waves ascribed to myocarditis.* (Record no. 616/48, leads I—III.)

a. The 3rd week of illness: perisinus rhythm with a frequency of 85 to 90. Pre-excitation is indicated by a short P-Q interval (0.10 sec.) and obliquely rising R waves in leads II and III. T_1 is slightly positive, T_2 and T_3 positive.

b. The 6th week of illness: sinus rhythm in leads I and II, perisinus rhythm in lead III with a frequency of 85 to 100; the P-Q interval is 0.12 to 0.14 sec.; the QRS complex is broadened and splintered in leads II and III; the T waves are positive.

c. Perisinus rhythm with a frequency of 80—100 later in the disease. The P-Q interval is 0.14 sec. in lead I; it is 0.11 sec. in leads II and III, in which pre-excitation seems to be present. T_1 is flattened, T_2 and T_3 are diphasic, indicating a myocarditis.

d. Recorded at the follow-up examination 5 years later during deep breathing. The frequency is about 80 with transition from perisinus rhythm in the first beat to sinus rhythm in the second. The P-Q time is 0.15 to 0.17 sec. and the QRS interval 0.08 sec. T_1 and T_2 are positive, T_3 is diphasic.

Before analysing these electrocardiograms a short theoretical recapitulation of the pre-excitation phenomenon is in order. The hypothesis of an extra abnormal connection between the auricle and ventricle (Kent's bundle in certain cases) by means of which a portion of the ventricle is prematurely activated, seems to be generally accepted. On the whole this explains rather well the electrocardiogram of pre-excitation with its frequently constant P-J time and tendency to paroxysmal tachycardia. It has also found support in the findings of pathological anatomy (Wood et al. 1943; Öhnell, 1944). The earlier the abnormally conducted impulse reaches the ventricle in relation to the normally conducted one, the wider and more distorted the ventricular complex. The anatomy of this connection, especially as related to the bundle of His and its branches, is of course of great importance. Interference between the two impulses or "mixed systoles" take place. Since the two impulses are probably to a different extent able to activate and produce the ventricular complex even in the same patient different types of complexes may occur. With minor time difference between the impulses a slight change may be observed, as in the case illustrated by figures 44 a and 44 c, with

disappearance of the Q wave and in its place an obliquely rising R wave. The ventricular complex is otherwise unaltered, and the prolongation of the QRS complex and the corresponding shortening of the P-R interval may amount to only a few hundredths of a second. A high-grade premature impulse on the other hand (figure 43) probably is able to take over the entire activation with consequent complete alteration of the ventricular complex. Kossman and Goldberg (1947) feel that this may explain why also the P-J time may be shortened in the presence of a very short P-Q time.

It has been generally observed that stimulation of the vagus favors and stimulation of the sympathetic system hinders pre-excitation. This is explainable on the basis of their different effects on the conduction system. In addition, when the vagus is stimulated a perisinus rhythm appears in many individuals, and it would seem likely that the somewhat altered route which the impulse then follows through the auricle may sometimes be of significance with regard to whether pre-excitation develops or not. On the other hand, an auriculoventricular nodal rhythm will be accompanied by a normal QRS time, since the conduction time over the ordinary route is then implying the shortest possible connection. As with ectopic impulse-giving centers and their manifestation, the time factor is decisive — the impulse which arrives first provides the activation. If the sinus impulse through the abnormal auricle-ventricle connection arrives before that passing over the bundle of His, pre-excitation occurs.

In the patients second electrocardiogram (figure 44 b) the P-Q time appears to be normal. Thus, a normal ventricular complex should be present, on the condition, of course, that atrioventricular as well as intraventricular conduction is intact. If the P-Q time is prolonged, pre-excitation may be present even with a normal P-Q interval. This would seem to be a possible situation in this instance, since the P-J time is prolonged from 0.22 to 0.26 seconds. In addition, the first portion of the R wave has the same appearance as in the complexes with pre-excitation. The middle portion of the ventricular complex is splintered, while the latter portion is practically unaltered and was probably released via the ordinary conduction pathways. Judging from the P-Q and P-J times in the patient's series of electrocardiograms, the normally conducted impulse would then have been more delayed than the premature one. The time difference between the impulses would thus be increased, which is in accordance with the widening and splintering of the QRS complex. It seems therefore posssible that pre-excitation may be present although with a greater time difference between the impulses than previously. The author considers that a less likely possibility is that an intraventricular conduction disturbance had developed.

This prolongation of the atrioventricular conduction time lies on the borderline of normal variability. In addition to this, however, the patient had an abnormal flattening of the T wave in leads II and III (figure 44 c) apparently unrelated to the pre-excitation. Judging from the follow-up examination it is possible that the pre-excitation was also associated with the scarlet fever. The flattening of the T wave supports a diagnosis of myocarditis in any case.

A 9 year old girl (record no. 4263/48), aside from the pre-excitation displayed evidence of myocarditis consisting of significant alterations in the S-T segment and the T wave also in the normal ventricular complexes. Pre-excitation appeared for the first time 2½ months after the onset of scarlet fever and was later seen again on a single occasion in a work electrocardiogram (figure 38 b). It is of course possible, although not apparent from the course of the illness, that a myocardial lesion was also the root of the pre-excitation.

In 2 adult patients a constant, short P-Q time in which the P wave passed directly into the QRS complex without any P-Q interval has been observed. The QRS complexes were of normal configuration, and for this reason the author has not felt that they should be classified as pre-excitation.

Summary of Intraventricular Disturbances

Judging from our experience with these 2831 cases using the standard extremity leads, it would seem that lesions of the intraventricular conduction system are not common in scarlet fever. A relationship between the scarlet fever and the conduction disturbance would seem to have existed only in the cases of 2 children who had intermittent block of the Wilson type. On the other hand, constant disturbances of intraventricular conduction, possibly on a congenital hereditary basis, have been observed in 27 patients. In addition, in 7 patients pre-excitation has been observed. This was in 5 cases a constant phenomenon and obviously not related to the scarlet fever.

CHAPTER 13

Survey of Electrocardiographic Changes

In table 8 a summary is given of the more important electrocardiographic changes observed, including whether these are regarded as indicative of a scarlatina myocarditis or not. The changes are divided into two main groups. Group I includes electrocardiographic changes which in themselves are considered to be evidence of an acute myocarditis during the scarlet fever. Group II contains changes of questionable nature, probably physiological variations, or consistent aberrations from the normal without demonstrable connection with the scarlatina. The latter group has been divided into a sub-group A, in which cardiac complaints indicated that there was an acute myocarditis during the scarlet fever, and a sub-group B without heart symptoms. Patients in group I are classified as myocarditis cases and in sub-group II A as "probable myocarditis cases". In the comparison between treatment series and in the statistical calculations chief regard has been paid to group I. Of course a patient's subjective complaints may be the only evidence of disease. It would seem, however, to be extremely doubtful whether we are justified in basing a differentiation on symptoms which must be burdened with appreciably greater sources of error than the electrocardiographic evaluation.

TABLE 8

Review of the Electrocardiographic Changes

	Changes in the P waves	Coronary sinus and A-V nodal rhythms	Ectopic systoles	A-V block	Changes in the T_{1-2} waves	Changes in the S-T segment	Disturbances of intraventricular conduction
I Changes regarded as myocarditis	1	8	4	34	59	11	2
II A Equivocal changes with symptoms: "probable myocarditis"	—	3	2	1	9	4	—
II B Equivocal changes without symptoms	44	32	41	42	50	20	27

CHAPTER 14

Factors which Influence the Incidence of Myocarditis

Introduction

Before a comparison of the various series can be made, it must be determined which factors other than treatment influence the incidence of myocarditis. It must be established that the different series to be compared are similar in these respects. Among such factors in scarlet fever are the geographical locality, the character of the epidemics, and possibly different seasons. The relation to the age and sex of the patients is to be studied and taken into consideration.

The interpretative standards, the frequency of electrocardiographic examinations and methods constitute also factors, which may markedly influence the number of myocarditis recorded. As shown by Holzmann (1937), among others, regular control recordings with the precordial leads during the course of an infectious illness will also show a greater number of pathologically changed T waves than the extremity leads. In addition there are probably cases of myocarditis which, as a result of their location, do not appear in the electrocardiogram even with our refined modern techniques.

The uniformity of parallel series

The geographic factor is constant, and during this period of observation the epidemics have been of a mild character with only isolated toxic cases. The patients have, of course, been assigned to the various series without regard to severity or other factors with the single exception that siblings have generally been assigned to the same series. Minor variations in the character of the scarlet fever may, however, have occurred from one time to another in relation to the seasons, but since the series have been concurrent even these variations have been similar in the penicillin and control series. Definite conclusions with reference to the effect of treatment are drawn only between parallel series. Certain comparisons are also made between combined penicillin series, from two or three periods, and concurrent control series. This is done only for special reasons and is clearly indicated.

Uniformity of the methods and interpretation

The methods and number of electrocardiograms are uniform in parallel series as reported. Since the author has evaluated all electrocardiograms herself, even the source of error which the electrocardiographer constitutes should have the same effect in all series. In addition, the electrocardiograms have been evaluated according to the record number without knowing at the time to which series they belonged. Any influence of the interpretation to the advantage or disadvantage of a particular series would therefore probably be excluded.

Influence of the Frequency of Recordings. Alternate-Day Electrocardiograms

It has been stated in the literature that the observed incidence of myocarditis will depend upon the number of the electrocardiographic recordings. It has also been observed several times in this study that even severe changes in the electrocardiogram, for example total A-V block, may be transitory and demonstrable only once. In order to obtain an idea of the difference which more frequent electrocardiograms would make, we have made recordings approximately every-other-day on a smaller number of patients, 208. At least 9 electrocardiograms per patient were taken during a three week stay in the hospital. Wickström had the same frequency of recordings in the 100 patients which he studied in 1932 although the hospital confinement was at that time considerably longer than now. The total number of recordings per patient was therefore larger in his study.

These so-called "alternate-day" electrocardiograms were taken in 1948, the majority of patients belonging to the P_2, P_5 and C_2 series. The alternate-day electrocardiograms were examined without reference to the routine electrocardiograms. On the basis of these more frequent recordings it was possible to make a diagnosis of myocarditis in 4 cases in which the routine electrocardiogram showed only doubtful changes, if any. One of these patients belonged to the P_2 series, one to the C_2 series, one to the desquamation group, while the other was not included in any series. These cases of myocarditis have, of course, not been included in the comparisons between the different series since the findings were made in departures from the usual procedure. In all of these cases there was only a mild change involved — 3 times a lowering of the T wave and in one case a slight prolongation of the P-Q time.

Other changes of not definitely pathological nature appeared in a number of cases. For example, coronary sinus rhythm was observed 3 times, premature systoles in 2 cases, and perisinus rhythm was seen several times. Thus, in this group of patients who through the usual procedure were found to have an incidence of myocarditis of 3.9 per cent (8 cases out of 208), the alternate-day

electrocardiograms resulted in an increase to 5.8 per cent which is far from unimportant. This experience would make one consider that all of our incidences of myocarditis are relative and dependent to a large degree on the frequency of electrocardiographic recordings. In our first series, P_1 and C_1, only 2 or 3 electrocardiograms were recorded for each patient during a 4—6 weeks period of hospital confinement. These 2 series may be compared with each other but not with the later series in which electrocardiograms were regularly recorded once a week.

Interpretation of the Degree of Severity

The degree of severity in addition to the actual number of myocarditis is important. Therefore, in addition to the total number of cases of myocarditis, the incidence of more severe cases has also been used as a basis of comparison between the different series.

The electrocardiographic changes have been taken as a starting point in evaluating the severity of the case. First, they provide an objective basis for a uniform selection in the different series. In addition, the electrocardiogram, despite its inadequacies, is the best basis for making a diagnosis of myocarditis. If clinical signs or symptoms were used, the evaluation would undoubtedly be more dependent on coincidence since these symptoms are markedly related to whether or not the patient is confined to bed and the burden on the heart thus lessened. The record would also be subjectively modified both by the patient and the evaluator to a greater extent.

The magnitude of the electrocardiographic alteration depends on the *location* of the lesion and is far from being consistently proportional to the *extent* of the lesion. The electrocardiogram reveals, by means of the electrical potentials which reflect impulse origin, conduction, depolarization and repolarization, the manner in which and the extent to which the myocarditis is disturbing some essential functions of the heart. Thus the electrocardiographic changes commonly parallel the clinical picture and course which are indeed the most important considerations. Certain changes have been found by experience in this and other studies usually to be drawn-out and associated with clinical symptoms, as for example inversion of the T wave, and other changes constitute an immediate threat to the function of the heart, such as in high-grade A-V block. It should, therefore, be justifiable to classify these changes as "severe" as to type and degree. These cases with more marked electrocardiographic changes would also be regarded as definitely pathological even by authorities with particularly strict criteria.

Among "severe" electrocardiographic changes it is necessary to eliminate groups in which the majority of the cases appear to be clinically mild, although isolated severe cases may be seen. Such sources of error should, however, be uniformly distributed in the different series and are unlikely to distort the results of a comparison.

TABLE 9

Incidence of Myocarditis in the Various Series

Series	Total cases of scarlet fever	Cases of myocarditis	
		No.	% of total
P_1	228	5 (6)*	2.2 (2.6)*
C_1	221	7 (8)	3.2 (3.6)
P_2	222	5 (6)	2.3 (2.7)
P_5	211	12 (12)	5.7 (5.7)
C_2	219	14 (20)	6.4 (9.1)
P_{pk}	368	15 (20)	4.1 (5.4)
P_{po}	339	12 (16)	3.5 (4.7)
C_3	99	6 (6)	6.1 (6.1)
O	306	16 (19)	5.2 (6.2)
D	505	13 (14)	2.6 (2.8)
I	113	5 (5)	4.4 (4.4)
Total	2,831	110 (132)	3.9 (4.7)

"P" indicates cases treated with penicillin; "C" controls; Concurrent series are grouped together by braces.

"O" patients not included in any series; "D" cases with desquamation; "I" cases of scarlet fever complicated by other infectious diseases (see page 13 and following).

*) Figures in parentheses represent number of cases of myocarditis if the "probable myocarditis" cases (page 23) are included.

With regard to *disturbances of atrioventricular conduction*, grades II and III block, even when transitory, have been included since they indicate a serious disturbance. With regard to grade I block, the basis of classification must be more complicated since it includes both severe and very mild changes. The degree of prolongation and the duration have been accepted as indicative. A prolongation of at least 0.08 seconds and at least one week's duration have been taken as the limit for predicating "severe" alterations. Actually, one of these cases, a 30 year old woman with a prolongation of the P-Q time of from 0.18

TABLE 10

Incidence of Myocarditis in Adults and Children

Series	Adults			Children		
	Total cases of scarlet fever	Cases of myocarditis		Total cases of scarlet fever	Cases of myocarditis	
		No.	% of total		No.	% of total
P_1	52	3	5.8	176	2	1.1
C_1	36	5	13.9	185	2	1.1
P_2	33	2	6.1	189	3	1.6
P_5	18	3	16.7	193	9	4.7
C_2	28	6	21.4	191	8	4.2
P_{pk}	18	3	16.7	350	12	3.4
P_{po}	17	2	11.8	322	10	3.1
C_3	2	—	—	97	6	6.2
O	26	3	11.5	280	13	4.6
D	43	5	11.6	462	8	1.7
I	6	—	—	107	5	4.7
Total	279	32	11.5%	2.552	78	3.1%

seconds to 0.30 seconds for a period of two months, was remarkably free from symptoms. However, it is noteworthy that with these disturbances a patient may be quite asymptomatic but suddenly develop serious symptoms if the block increases. A number of these patients have experienced cardiac symptoms such as dyspnea and precordial pain, and decompensation has also been observed.

Only inversions unrelated to tachycardia (group A, page 125) have been considered in regard to changes of the T wave. As a rule, it is possible to follow the regression of these changes through the smaller stages — isoelectric and lowered T waves — which certainly correspond to less serious lesions in general. The clinical course has in most cases confirmed the presence of a more severe disturbance and the regression has taken place slowly.

Survey of the Different Series

Before making any conclusions regarding the incidence of myocarditis, a survey of the series, including the total number of myocarditis cases in each, is

TABLE 11

Distribution of Myocarditis in Adults and Children with Regard to "Severe" Electrocardiographic Changes

Series	Adults			Children		
	Per cent of total cases of scarlet fever			Per cent of total cases of scarlet fever		
	Severe*)	Total	Probable	Severe*)	Total	Probable
P_1	0	5.8	(5.8)	0	1.1	(1.1)
C_1	*5.6*	13.9	(13.9)	0	1.1	(1.1)
P_2	0	6.1	(6.1)	*0.53*	1.6	(2.1)
P_5	*11.1*	16.7	(16.7)	0	4.7	(4.7)
C_2	*14.2*	21.4	(21.4)	0	4.2	(7.3)
P_{pk}	0	16.7	(16.7)	*0.29*	3.4	(4.9)
P_{po}	*5.9*	11.8	(11.8)	*0.31*	3.1	(4.3)
C_3	—	—	—	0	6.2	(6.3)
O	*7.7*	11.5	(11.5)	0.36	4.6	(5.7)
D	*7.0*	11.6	(14.0)	*0.22*	1.7	(1.7)
I	—	—	—	*0.93*	4.7	(4.7)
Total	*5.0%*	11.5%	(11.8)%	*0.24%*	3.1%	(3.9)%

*) "Severe" electrocardiographic changes are italicized.

Figures in parentheses represent the number of cases of myocarditis if the "probable myocarditis" cases (page 23) are included.

See table 9 and 10 for total cases of scarlet fever and of myocarditis.

offered in table 9. (See also table 1.) Of the 2,831 patients, myocarditis has been diagnosed on the basis of eletrocardiographic changes in 110, corresponding to an average incidence of 3.9 per cent. In another 22 cases a diagnosis of "probable myocarditis" has been made on the basis of equivocal electrocardiographic changes associated with subjective heart complaints (page 23). If the latter are included in the total, the average incidence of myocarditis becomes 4.7 per cent. The incidence in adults and children, as well as the distribution of the severe alterations, is given in tables 10 and 11.

As mentioned above, electrocardiographic recordings were made fairly infrequently in the two first series, one with injected penicillin, P_1, and one control series, C_1. The incidence of myocarditis in these groups is also relatively low — 2.2 and 3.2 per cent respectively. In the P_1 series, there are no cases with severe alterations of the electrocardiogram, while in the C_1 series were 2 severe cases, both adults. An 18 year old woman (record no. 2496/47, page 90) had grade II block with Wenckeback periodicity and a prolonged illness suggesting a complicating rheumatic fever. A 37 year old man (record no. 1860/47) displayed inversion of the T wave.

During the next period there were three parallel series. Group P_2 received intramuscular penicillin starting on the second hospital day and P_5 beginning on the 5th day. The control group, C_2, were cared for on isolation wards. In group P_2 there was an overall incidence of myocarditis of 2.3 per cent with only one severe case. This was a 12 year old boy (record no. 4289/47, page 182) with a prolongation of the P-Q time from 0.13 seconds to 0.22 seconds. (It is worth noting that a few years earlier, he had been admitted to hospital with a diagnosis of tonsillitis and acute myocarditis. At that time he was found to have a prolongation of the conduction time and would thus seem to have had a tendency to this type of change prior to scarlatina.)

In series P_5, there were 5.7 per cent who showed electrocardiographic changes of which 2 were adults with "severe" changes. A 39 year old woman (record no. 4821/47, page 125) developed a protracted inversion of T_1 and T_2, and a 20 year old man (record no. 1042/48, page 67) was found at the follow-up examination in the sixth week to have developed a new hemolytic infection with synovitis and to have P-Q prolongation and extrasystoles from two foci.

The greatest number of "severe" cases, all adults, is to be found in Group C_2 which had a myocarditis incidence of 6.4 per cent (9.1 per cent if "probable" cases are included). One of these patients had synovitis as well as a total A-V block in the electrocardiogram plus an isoelectric T wave (record no. 147/48, page 90) and another had a persistently negative T wave (record no. 34/48). The others were a 15 year old boy (record no. 3662/47) with a P-Q prolongation following a new throat infection and a 21 year old woman (record no. 407/48, page 126) with otitis and inversion of the T wave.

During the next period, 1948—1949, it was possible to continue the control series, C_3, only for a shorter period because of technical difficulties. It is considerably smaller than the P series and not entirely comparable with the latter. C_3 included 6 per cent with mild electrocardiographic changes, all in children. Group Ppk received procaine penicillin per injection, while those in Ppo were given amorphous penicillin by mouth. There was an incidence of 4.1 per cent myocarditis in group Ppk. With one exception, a 5 year old girl (record no. 3653/48, page 93) with persistent prolongation of the PQ time, the alterations were, however, mild. In series Ppo a 17 year old girl (record no. 331/49) displayed a grade I A-V block, while a 6 year old boy (record no. 1776/49, page 94) developed a persistent prolongation of the P-Q time.

Group O consists of patients not included in any of the other series for reasons mentioned in Chapter I. The majority of these were also treated with penicillin early in the course of the illness. Two women (record nos. 363/49 and 4475/47) had conduction disturbances of a more severe type. They received penicillin from their first and second days in the hospital respectively. An 8 year old girl (record no. 3014/48, page 108) displayed inversion of T_2.

Among the desquamators myocarditis occurred in 2.6 per cent. The relatively low incidence probably depends on the fact that they were not observed during the early part of the illness, thus providing time for possible alterations to have regressed. On the other hand, a number of severe alterations were observed. Three patients (record nos. 3616/47, 1888/48, and 1132/48) had negative T waves. An 11 year old boy (record no. 841/49, page 91) developed

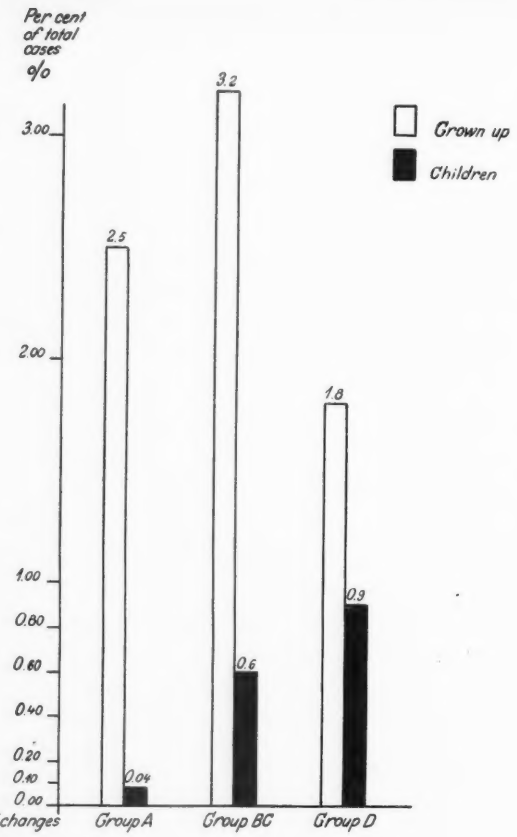


Fig. 45. *Changes in the T waves in adults and children.*

Group A contains negative, group BC isoelectric and diphasic, and group D lowered T waves. The incidence is given as a percentage of the total number of adults (279) or of children (2552). The incidence of all degrees of changes is higher in adults, the difference is greatest in the more marked changes.

a grade II A-V block of short duration in the seventh week in connection with a new hemolytic infection.

In group I, in which other infectious diseases were present along with scarlatina, the incidence of myocarditis was 4.4 per cent. Only one case with a more "severe" A-V conduction disturbance was observed (record no. 883/49, page 123).

The Influence of Age

The patients have been divided, according to age, into two groups, adults and children. All who reached the age of 15 during their year of admission or were

older have been considered as adults. As expected, the adults are in a considerable minority — 279 as against 2,552 children. Table 10 shows how the adults and children are distributed with regard to the various groups and incidence of myocarditis. It is particularly striking that the adults have a much higher incidence of myocarditis, 11.5 ± 2.0 per cent, than the children, 3.1 ± 0.3 per cent. This difference, 8.4 ± 2.8 per cent, amounts to more than three times the average error and is statistically significant. It is clearly apparent in all series.

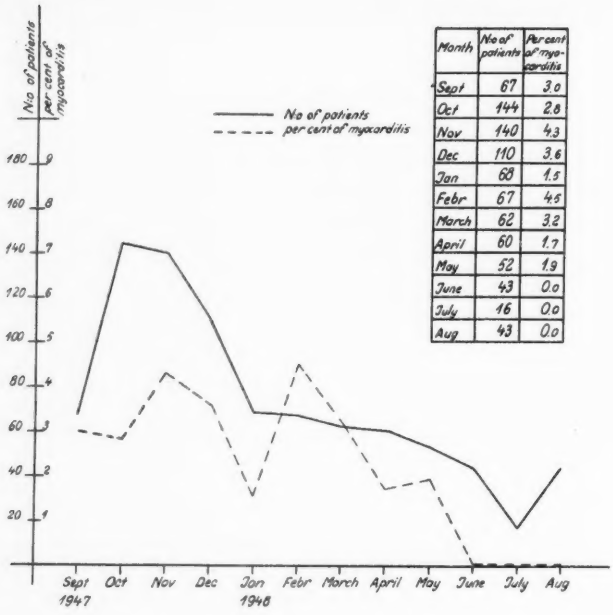
The difference is also marked with regard to "severe" electrocardiographic changes (table 11). The incidence is seen to be 5.0 ± 1.3 per cent in adults as against only 0.24 ± 0.09 per cent in children, the difference thus being 4.8 ± 1.3.

Another classification has also been used which is based on changes in the T waves. These were the commonest indication of myocarditis in this study, and significant alterations have been observed in a total of 59 patients. According to the type of change they may be classified in groups which reflect the magnitude of the alteration and which may be used as a basis for analysis. The most marked changes with negative T waves have been classified under group A. The somewhat milder forms with isoelectric or diphasic T waves have been included in group BC, while the least marked of all, consisting simply of a lowering of the T wave, have been assigned to group D. (See also pages 123 and following.) Justification for this type of classification is found in the fact that the duration of the change is generally longer for the more pronounced changes.

With regard to the T wave changes, as with regard to myocardities in general, the incidence is higher among adults (7.9 per cent) than among children (1.8 per cent). The difference, 6.1 ± 1.6 per cent, is statistically significant. All types of T wave changes are commoner among the adults as is to be seen in figure 45 which also shows that the difference is greatest in the more marked changes, and diminishes with the degree of the alteration. Thus in group A (negative T waves) the incidence is 2.5 per cent for adults as compared to 0.04 per cent for the children, while in group D (flattening of the T wave) the adults' incidence is 1.3 per cent compared to the children's 0.9 per cent. Although this classification of the T wave changes is rough and schematic it shows in a striking way that there is not only a greater likelihood of myocarditis developing in older patients but also a likelihood of more marked changes appearing. Consequently, it is necessary to consider the children and adults separately when comparing the different series.

The Influence of Sex

In addition to the age factor the significance of sex has been studied. A somewhat higher incidence of myocarditis was observed in the girls (3.3 per cent) than in the boys (2.9), which agrees with previous reports in the literature. (See Steinmann 1945 and his bibliography.) In adults the men had a higher incidence than the women — 13.9 contrasted with 9.8 per cent. None of these



Month	No of patients	Per cent of myocarditis
Sept	67	3.0
Oct	144	2.8
Nov	140	4.3
Dec	110	3.6
Jan	68	1.5
Febr	67	4.5
March	62	3.2
April	60	1.7
May	52	1.9
June	43	0.0
July	16	0.0
Aug	43	0.0

Fig. 46. *Variations in monthly number of scarlet fever cases in children and corresponding incidence of myocarditis during a year.*

differences has been statistically established, however. In comparing the different series it would therefore be possible to disregard this factor without introducing any significant error.

The Influence of Seasonal Variations

In order to determine whether or not any seasonal variation occurs, the incidence of myocarditis was calculated for all patients during the various months from September 1947 through August 1948. The highest incidence was during the winter. The lowest was during the summer, when the total of patients with scarlatina was also lowest (figure 46). Since the incidence of myocarditis is higher among adults than children (see page 163) they have been compared individually. The various treatment series are evenly represented within the various months, and for this reason no subdivision has been attempted in this regard.

Since the adults were so few in number, 118 during the entire year, it was decided to compare the winter half of the year, October through March, with the summer half, April through September. The incidence was 19.3 per cent (16 out of 83) as compared to 5.7 per cent (2 out of 35). This difference

13.6 ± 5.8 per cent, which amounts to nearly two and one half times the average error does not justify any definite statistical conclusions but indicates a real variation.

With regard to the children the incidence was 3.4 per cent (20 out of 591) during the winter half as against 1.4 per cent (4 out of 281) during the summer half. The difference, 2.0 ± 1.0 per cent, does not reach the level for probability. The difference would be expected to be least during the months which are near the seasonal change and greatest at the middle of the periods. For the winter months, November through February, the incidence was 3.6 per cent (14 out of 385) and for the summer months, May through August, 0.7 per cent (1 out of 154). This difference, 2.9 ± 1.2 per cent, is almost probable. The desquamators have been included in the comparison since the seasonal variations would probably be reflected also in their case and since they constitute an approximately equal proportion of the patients from the two periods (23 and 21 per cent respectively).

Only a comparison between a number of years can determine whether the variation found in the incidence of myocarditis is due to chance variations in the character of the current form of scarlet fever or is consistently associated with the time of the year. This observation illustrates the need for strictly coterminous series if conclusions are to be drawn with regard to the results of treatment in myocarditis.

Intrinsic Effects of Penicillin

An extremely important question which must be considered is whether penicillin itself may produce electrocardiographic alterations. So far as is known, the only effort to answer this question was made by Trautmann on a group of 65 patients with gonorrhea. On comparing the electrocardiograms taken before and after 24 hours of intensive penicillin treatment, he found variations in the conduction time, even without frequency changes, of as much as 0.05 seconds. Supraventricular extrasystoles were found in one case, while in a few other cases previously present nodal rhythms, wandering pacemakers, and ventricular extrasystoles disappeared. Trautmann regarded the alterations observed as explicable on the basis of vegetative changes. The value of this study is probably somewhat diminished by the fact that there is a possibility of myocarditis occurring in such patients. Thus it is theoretically possible that the electrocardiographic changes might be manifestations of myocardial involvement. A similar study on entirely healthy subjects would be desirable because only such an experiment could show a pure penicillin effect. In practice, however, the effect of penicillin on the myocardium during an active infection is of chief importance, and this is an extremely difficult factor to analyze.

Consequently we do not at present know the effect of penicillin on the electrocardiogram. In this work, however, an immediate effect due to vegetative factors is of minor importance since an effort has been made to identify electrocardiographic changes resulting from vegetative factors and because early myocarditis is of lesser practical importance anyway. In making certain calculations in the present study such changes have been omitted. It is possible that an allergic reaction to the penicillin might, however, produce a *late* myocardial damage effect. Underlying the development of a myocarditis there would seem to be a complex mechanism in which allergic reactions probably play a part. Penicillin is now known for its limited tendency to produce side effects, and it relatively seldom produces hypersensitivity reactions.

Each injection probably involves some trauma to the tissues, but it is not known whether this has any significance. This factor can be dismissed in the Ppo series where oral penicillin was administered. In the parallel Ppk series patients received injections of procaine penicillin. The concentrations of penicillin in the serum, the effect of the penicillin with regard to the disappearance of the hemolytic streptococci from the nose and throat, and the bacterial complications in these series have been studied by Lagercrantz and Peterson (1950) and Ström (1950). From these studies it may be seen that the serum concentration, both with parenteral and oral administration, varies markedly from one individual to another but is significantly greater with parenteral administration. By this route a concentration was obtained which was calculated to be effective against the hemolytic streptococci in the mucous membranes of the nose and throat for twice as long as when the drug is administered orally.

In regard to the disappearance of hemolytic streptococci the results were also appreciably poorer with oral than with hypodermic administration. During the first period of study, when a lower dosage was used, the results in both groups were unsatisfactory in this respect. In the orally treated group there was curiously no apparent correlation between bacterial relapses and serum concentration. It is noteworthy that with regard to bacterial complications there were no clinically clear differences either with respect to the different routes of administration or dosage.

As to the incidence of myocarditis, the number of adults affected was so small that it was impossible to draw any conclusions. Among the children the incidence was 2.8 per cent (7 cases out of 254) in the low dosage series Ppk, while in the concurrent Ppo series it was 2.1 per cent (5 cases out of 242). In the later higher dosage Ppk series the incidence was 5.2 per cent (5 cases out of 96) and in the Ppo series 6.3 per cent (5 cases out of 80).

Thus there was no noteworthy difference in incidence with regard to the various modes of administration, and with regard to the various dosage levels there was a higher incidence of myocarditis with the higher dosage despite a higher serum concentration of penicillin and a better bacteriological result. The

difference was not great enough for statistical significance, however. The possibility of a variation dependent on the time of year or differences in the character of the bacterial strain from time to time must be left open for consideration. In conclusion it may be said that both methods of treatment would seem to be equal with regard to the incidence of myocarditis. However, in addition to the route of administration, both the different preparations and their serum concentrations may have contributed to this equality of result.

CHAPTER 15

Comparison of the Cases Treated with Penicillin and the Controls

In comparing the P series, it must be remembered that the possible effect of penicillin on the incidence of myocarditis may depend on various factors possibly having different effect. The final clinical result is, of course, the important thing in regard to our therapeutic procedure. (An improvement in therapy would, however, be possible only after an analysis of the effects of such various factors.)

The Series P_1 and C_1

The patients in the two first series, P_1 and C_1, underwent fewer electrocardiographic studies than the others, and a lower number of recorded myocarditis cases must therefore be expected. Between themselves, however, the two series are fully comparable. The dosage of penicillin was increased at approximately the midpoint in the series since the hemolytic streptococci were not disappearing with the rapidity which for practical reasons was desired. However, even with the lower dosage the clinical results must be regarded as good with a nearly complete disappearance of the bacterial streptococcal complications (Ström, 1948). It should therefore be fully justifiable to consider the series as a unit with regard to the clinical effect of penicillin. In confirmation of this the penicillin effect, based on the disappearance of the hemolytic streptococci, was found to be adequate when the only two cases of myocarditis which developed during the lower dosage period were evaluated.

Adults: The incidence of myocarditis in the penicillin series was 5.8 per cent as against 13.9 per cent in the control series. However, the number of cases is small, and the difference of 8.1 ± 6.7 per cent does not permit the drawing of any conclusions. With regard to "severe" electrocardiographic changes the incidence was 0 in P_1 compared with 5.6 per cent in C_1, the difference being 5.6 ± 4.3 per cent.

Children: The incidence here was the same in both series, 1.1 per cent.

The Series P_2, P_5, and C_2

In the P_2 and P_5 series in which treatment with penicillin was begun on the second and fifth hospital days respectively, we went from 3 injections per day

during the first period to 2 injections per day. In this study, as in others concerning scarlatina, this schedule was found to be sufficient to achieve the full penicillin effect, and the series have therefore been compared in their entirety. The patients in the concurrent control series, C_2, were confined in isolation rooms.

It may be asked at this point what importance isolation can have in regard to the incidence of myocarditis. Lichtenstein (1931) and Bergman (1944) have previously carried out studies in this hospital on the influence of such isolation on the incidence of various complications of scarlet fever. In the earlier study there were no cases of myocarditis, while the latter one showed that although the septic complications diminished under isolation the incidence of toxic complications was unaltered. Thus myocarditis occurred in 5 per cent of the isolated patients compared to 3 per cent of the ward patients. A noticeable difference in the incidence of myocarditis in control series C_2 could not, therefore, be expected had these patients been cared for on the wards.

Adults: The incidence of myocarditis is lowest with early penicillin treatment, 6.1 per cent; highest in the control series, 21.4 per cent; while in P_5 it was 16.7 per cent. The difference between P_2 and C_2 is 15.3 ± 8.9 per cent and thus fails to amount to as much as twice the average error. The relationship is similar with regard to "severe" alterations of the electrocardiogram. These are entirely absent from P_2 but appeared in 11.1 per cent in P_5 and 14.2 per cent in C_2. The difference here between P_2 and C_2 is 14.2 ± 7.4 per cent and thus amounts to nearly twice the average error. Of course, it is hardly to be expected that statistically significant differences could be brought out with such a limited number of cases.

The tendency to a decrease in the incidence of myocarditis as well as of more severe alterations in the electrocardiogram with penicillin treatment is fully demonstrated, however, and in agreement with the preceding period. These periods are not entirely comparable due to the less frequent electrocardiographic studies in P_1 and C_1 which should be accompanied by a decrease in the number of observed myocarditis cases. This is also in agreement with the lower figures for the earlier series. The difference found is also less. It would therefore seem permissible to compare the series together since the source of error to be considered in P_1 and C_1 (which as a result of the larger number of adults have the greater effect on the values) should, if anything, decrease the actual difference.

The incidence in P_1 plus P_2 is 5.9 per cent (5 out of 85) and in C_1 plus C_2 17.7 per cent (11 cases out of 64). This difference, 11.8 ± 5.5 per cent, is not definitely established either. "Severe" electrocardiographic changes were lacking in P_1 plus P_2 (0 cases out of 85) but were present in 9.4 per cent of C_1 plus C_2 (6 cases out of 64). This difference, 9.4 ± 3.8 per cent, is statistically probable.

Children: Even in the case of children P_2 shows the lowest rate of incidence, 1.6 per cent, as against 4.2 per cent in C_2, the difference being 2.6 ± 1.7 per cent. There is thus no definite difference between the two groups. In group P_5 the

incidence is somewhat higher, 4.7 per cent. If the "probable myocarditis with equivocal electrocardiographic changes plus subjective cardiac complaints" (page 23) cases are included, the incidence increases to 2.1 per cent in P_2 as compared to 7.3 per cent in C_2. This difference, 5.2 ± 2.2 per cent, is nearly probable, but the author does not feel justified in drawing any definite conclusions from these values. There are no cases of "probable myocarditis" observed in P_5. "Severe" electrocardiographic changes are so rare in the children that they do not make a comparison possible.

With regard to the time that treatment was begun, the incidence is higher when begun late (4.7 per cent) than when it is begun early (1.6 per cent). This difference, 3.1 ± 1.8 per cent, is not statistically significant. Thus, there is also a tendency in children to a decrease in the incidence of myocarditis when penicillin treatment is begun early as compared with late treatment or none. These differences do not, however, come up to the statistically significant level although large series are involved.

The Series P_{pk}, P_{po}, and C_3

The two series, Ppk and Ppo, have been compared previously and discussed with regard to different ways of administering penicillin (page 166). As to the incidence of myocarditis, they are roughly equal with 3.4 per cent and 3.1 per cent respectively in children. As mentioned previously the control series, C_3, which accompanied Ppk and Ppo was incomplete, but it does, however, clearly show the same tendency displayed in the earlier periods, i. e. a higher incidence in the controls — 6.2 per cent or 6 out of 97 cases. The difference between C_3 and Ppo, 3.1 ± 2.6 per cent, is also insufficient to permit any conclusions.

The Rest of the Material

Group 0 is a heterogeneous group in which many patients suffered from complications even at the time of admission. It also extends over a long period of time. The incidence, 11.5 per cent (3 out of 26) in adults and 4.6 per cent (13 out of 280) in children, lies between the P and the C series but is closer to that of the controls. This is also the situation with *group I* in which other infectious diseases were present and which includes cases both with and without penicillin treatment as well as some in the desquamating stage. The greatest majority of these were children. Their incidence of myocarditis is 4.7 per cent (5 cases out of 107) while in the other children the average incidence is 3.0 per cent (73 out of 2,445). The difference is 1.7 ± 2.0 per cent. There is thus no obvious tendency on the part of the infections involved, which were predominantly of a virus nature, to increase the incidence of myocarditis.

The *desquamators* are comparable with the control cases in view of the fact that they did not receive penicillin until late in the illness, if at all. Due to the

abbreviated period of observation some cases of myocarditis have, of course, escaped diagnosis. Among the adults the incidence was 11.6 per cent (5 cases out of 43), thus lying somewhat under the level of the control cases, while in the children the frequency was 1.7 per cent (8 cases out of 462) which lies at the same level as the penicillin-treated cases. "Severe" electrocardiographic alterations are more likely to be discovered because they are often more persistent and frequently associated with clinical indications. The incidence of "severe" electrocardiographic changes was 7.0 per cent in the adults and 0.22 per cent in the children.

It would seem to be of interest to determine how large a percentage of the adults with myocarditis have displayed "severe" electrocardiographic alterations and to compare the incidence of myocarditis in the patients receiving early treatment with penicillin (P_1, P_2, Ppo, and Ppk) with that in those receiving penicillin only later, if at all (C_1, C_2, P_5, and D). These groups are actually not entirely comparable, but due to the fact that the adults are so few in number it would seem justifiable thus to obtain larger groups for comparison in order to see if the tendencies persist and become more definite. The lower frequency of electrocardiographic examinations among the desquamators is a factor which would probably result in the difference appearing to be smaller than it actually is. The first combined group has 1 "severe" case out of 10 cases of myocarditis in a total of 120 patients, or 0.84 per cent. The latter group has 11 "severe" alterations out of 19 myocarditis cases from 125 scarlatina patients, or 8.8 per cent. The difference is 8.0 ± 2.7 per cent and shows the previously observed tendency toward a reduction in the incidence of "severe" electrocardiographic changes in adults treated early with penicillin. The incidence of negative T waves in the former group is 0, in the latter 5.6 per cent (7:125). This difference, 5.6 ± 2.1 per cent, is probable.

Summary

Early treatment with penicillin in *adults* reduces the incidence of "severe" electrocardiographic changes with a statistical probability. Penicillin also shows a constant tendency to reduce the incidence of myocarditis throughout, although this has not been statistically confirmed.

In *children*, it has not been possible to demonstrate statistically significant difference as a result of early penicillin treatment in spite of the large series. There is, however, a general tendency toward a decrease in the incidence of myocarditis.

When the penicillin treatment is begun later a higher incidence of myocarditis in adults and children is found as compared with early treatment although the difference does not come up to a statistically significant value.

TABLE 12

Onset of Myocarditis in the Various Series

Series	P_2	P_5	C_2	P_{pk}	P_{po}	C_3	O	D	I*	Total
Total number of patients	222	211	219	368	339	99	306	505	91	2,360
Total cases myocarditis	5	12	14	15	12	6	16	13	4	97
Onset										
1st wk. Transitory	0	2	1	4	6	0	2	0	0	15
1st wk. Persistent	0	1	4	2	1	2	0	0	0	10
2nd wk.	2	2	2	6	1	0	5	4	1	23
3rd—4th wk.	2	2	8	1	3	1	5	5	1	28
5th—6th wk.	0	6	3	3	1	0	1	2	1	17
7th wk. or later	1	0	0	0	0	0	3	2	0	6
Indefinite								1		1

*) Only regularly examined cases are included.

Onset of Myocarditis

The time of onset of myocarditis has been compared in all of the regularly examined patients — 2,360 cases with 97 cases of myocarditis. The two earliest series, P_1 and C_1, have not been included because of the less frequent electrocardiographic recordings.

The three routine electrocardiograms during the hospital stay were usually made in the first, second and third weeks of illness. Only a few recordings were made during the fourth week since the patients had usually returned to their homes by this time. In occasional cases, however, the third recording was taken in the fourth week and a correspondingly smaller number of examinations were made in the first week. The isolated positive findings from the fourth week have been grouped with those in the third week. The first follow-up examination in the outpatient clinic has usually been made in the fifth week, although sometimes in the sixth; and findings from the fifth and sixth weeks have therefore been grouped together. The second outpatient check-up has been made in the seventh week or later.

The onset has been recorded within the following periods — first week of illness, second week, third to fourth week, fifth to sixth week, and seventh week or later. Each period thus generally corresponds to one electrocardiographic examination per patient, and the figures are with regard to this mutually comparable. When, according to the electrocardiogram, a myocarditis has regressed but a new alteration has subsequently developed, the onset of both processes has been recorded.

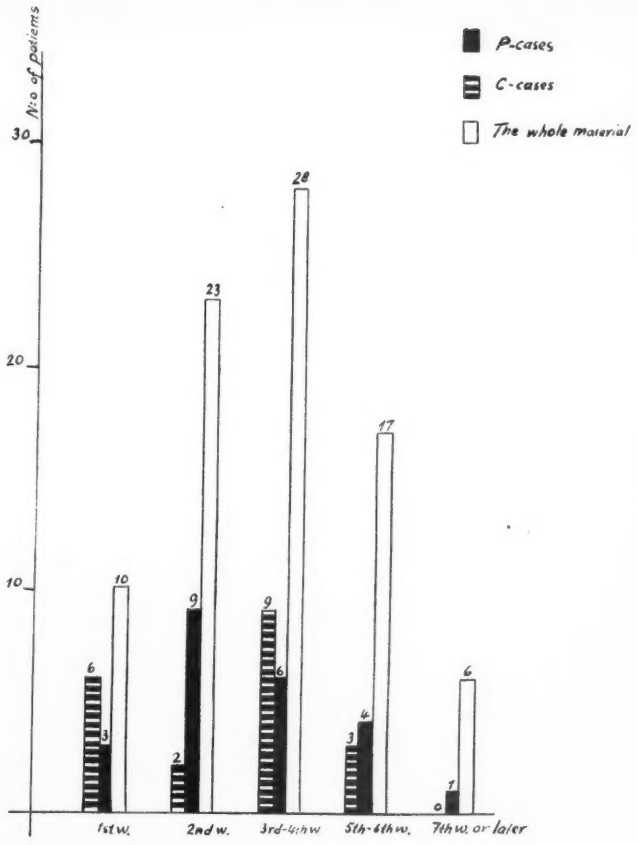


Fig. 47. *Onset of late myocarditis.* The total number of myocarditis cases are accounted for the whole material, for all cases given early treatment with penicillin, and for all controls.

Early and Late Myocarditis

Cases of myocarditis which made their appearance during the first week of scarlet fever have been divided into two groups according to their duration. The transitory ones which persisted for less than one week have been classified as "early myocarditis" ("Frühmyokarditis"). These are probably ol lesser practical importance and total 15 cases in this study. Those which persisted for a longer period of time have been regarded as "late myocarditis" ("Spätmyokarditis") and genuine scarlet fever myocarditis.

With regard to late myocarditis the relationship between the various series is the same as if the total number of cases of myocarditis were included in the

TABLE 13

Duration of Myocarditis in the Various Series

Series	P_2	P_5	C_2	P_{pk}	P_{po}	C_3	O	D	I	Total
Duration										
In only 1 recording	1	7	6	8	8	2	9	7		48
1 wk.	2	0	2	3	1	1	1	1		11
2—4 wk.	1	3	3	2	2	0	2	2		15
5—8 wk.	0	1	1	0	0	3	1	2	3	11
3 mo.—5 mo.	1	1	1	0	0	0	0	0		3
6 mo.—12 mo.	0	0	1	0	0	0	1	0		2
> 1 yr.	0	0	0	1	1	0	0	0		2
Indefinite				1			2	1	1	5
Total	5	12	14	15	12	6	16	13	4	97

calculations. Furthermore, there is no change regarding the statistical significance of the differences.

The onset of the myocarditis for all cases is shown in table 12. The maximum of late myocarditis is observed in recordings taken in the period "third to fourth weeks". The onset of late myocarditis for the early penicillin treated series (P_2, Ppk, and Ppo) compared with control series (C_2 and C_3) is shown in figure 47. Even with penicillin treatment the possibility of a myocarditis onset late in the course of the illness must be considered although the risk seems to be somewhat lesser.

Duration of Myocarditis

The duration of the electrocardiographic changes in rest is summarized in table 13. All of the regularly examined patients have been included in this summary also; there is thus a total of 97 cases of myocarditis. Since in these series there was no tendency to longer duration in adults than in children, all cases have been grouped together. Alterations which were observed in only one electrocardiogram have been noted under the column headed "In only 1 recording". Since the electrocardiograms were only recorded once each week it is impossible to offer any more exact report on the duration, but in these cases it was less than one week. When alterations have been present in two electrocardiograms the duration must have been at least 8 days, and they have been recorded in the column headed "1 wk". When regular examinations were impossible the duration has been recorded as "Indefinite".

The majority of the alterations were of short duration, and half of them were observed in only one electrocardiogram. One-fourth of the patients (26) have

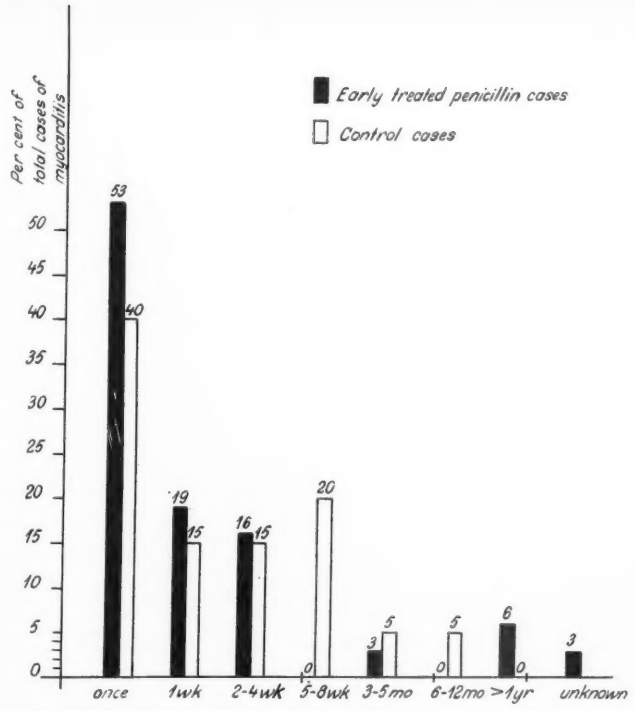


Fig. 48. *Duration of myocarditis in cases given early penicillin treatment as compared with in control cases.* The figures are given in percentage of total number of myocarditis cases.

had changes for from one to four weeks, and 11 patients for from five to eight weeks. Of the remaining 7 patients, 5 normalized within one year while 2 cases appeared to have developed permanent disturbances and are discussed in greater detail in the chapter on prognosis. The duration was indefinite in 5 cases.

With regard to the various treatment series, the duration for all cases treated early with penicillin (927 cases of scarlatina with 32 cases of myocarditis) and all control cases (318 cases with 20 of myocarditis) has been reported in figure 48. There is a tendency to shorter duration in the penicillin series than in the controls. The most striking divergence is in connection with the changes having a duration of from 5 to 8 weeks. These comprise 20 per cent of the cases of myocarditis in the controls and 0.0 per cent of the cases in the penicillin series. The difference, 20.0 ± 9.5 per cent, is not statistically probable. The difference with regard to changes persisting for more than 4 weeks is also not significant. Thus it may be said in conclusion, that there was a tendency to shorter duration of the myocarditis in the series treated with penicillin than in the controls.

CHAPTER 16

Discussion of the Various Factors, Particularly Penicillin Treatment, Affecting the Incidence of Myocarditis

Pathogenesis

Before commencing the discussion it seems advisable to say a few words about the pathogenesis of scarlatina myocarditis. This myocarditis is frequently subdivided into two types (see Steinman's monograph) — an early or so-called "Frühmyokarditis" and a late or so-called "Spätmyokarditis".

A "Frühmyokarditis" is connected with the initial fever stage and is regarded as being due directly to toxic factors. As a rule it is of only short duration and therefore of lesser practical importance, although it is possible that such a condition may continue into a "Spätmyokarditis". A "Frühmyokarditis" is generally manifested in the electrocardiogram by depression of the T waves.

On the other hand "Spätmyokarditis", the real scarlatina myocarditis, typically appears first in the second or third week of the primary illness. It may, however, develop even as late as the fifth, sixth, or seventh weeks. The toxin in this type is involved in a complex disease mechanism in which allergic factors probably play a part. In this group there is frequently A-V block, and in Steinman's material this was the commonest type of disturbance in cases of late myocarditis. In the author's material, however, T wave changes predominate both in the early as well as in the late types.

Importance of the Premorbid Constitution

Since an allergy factor probably plays a part in the pathogenesis of late myocarditis it is obvious that the patient's constitution is of importance. In addition to constitutional tendencies it is likely that infections which the patient has experienced earlier may be of importance. This hypothesis finds support in the fact that myocarditis is more commonly found in subjects who have had previous infectious diseases according to Wickström (1932) and that the degree of severity of the alterations in such cases is greater (Spang and Welsch 1947). This is thought to be due to the development of a hypersensitivity in certain parts of the cardiac muscle, and it is supposed that the same alteration may

reappear with subsequent infections — a phenomenon which was observed in 4 of the patients in this study. Concurrent infections of other types, frequently viral, which were present in 115 of these patients do not seem to have noticeably influenced the incidence of myocarditis (page 170), however.

It is obvious that if a hypersensitivity is present prior to the onset of the primary illness the "reaction time" for a scarlatina myocarditis of allergic etiology may be appreciably shortened. Thus alterations typical of late myocarditis, such as A-V block, may be manifested early in the illness. Of the 34 cases of A-V block in this study, 7 made their appearance during the first week. It is probably significant that the 2 patients with permanent disturbances of auriculoventricular conduction, as well as the patient found to have a latent conduction disturbance at follow-up examination, belong to the latter group. Two of these 7 patients displayed alterations for a long time — close to one year. One of them subsequently experienced a recurrence of grade II block. The sixth of these cases was found to be acutely ill with an elevated sedimentation rate at the time of a follow-up examination one and a half years later. It was discovered that he had an A-V block also at this time. The electrocardiogram again become normal afterwards. The seventh patient did not appear for follow-up examination.

It would thus seem that these early-appearing A-V blocks have a more serious implication with regard to prognosis than other disturbances of auriculoventricular conduction or alterations of the T waves. Those of the latter, even when of the late myocarditis type, which developed during the first week have, without exception, regressed. The fact that the auriculoventricular system is an anatomically demarcated unit for which nothing else can substitute and upon which great functional demands are made may of course be a factor in the prognosis. This factor must, however, influence all A-V lesions, so the serious prognosis with the early ones would seem to be a result of a premorbid change more than of a lesion entirely resulting from the scarlet fever.

Relationship to Rheumatic Fever

A relationship between the myocarditis of scarlet fever and that of rheumatic fever has been suggested by a number of workers (among others, Damade and Vergez 1947, Roelsen 1941) and by Watson (1945) in regard to late scarlatina myocarditis. This hypothesis finds a certain amount of support in the similar although not identical alterations found in pathological sections (Fahr, 1930). However, this does not necessarily imply, as Stoeber (1935) has pointed out, that they are the same disease.

The occurrence of synovitis in association with scarlet fever is of theoretical interest in regard to the above-mentioned relationship between scarlet fever and rheumatic fever myocarditis as well as with respect to the supposed allergic component in the pathogenesis of myocarditis. The disease picture with a streptococcal infection which is followed by an arthritis and myocarditis is quite uni-

form, and in the literature the term "scarlatina rheumatoid" is often found. The difference between this condition and rheumatic fever is supposedly more a question of degree than of type, and in association with scarlet fever a clinically unmistakable rheumatic fever sometimes develops.

Relationship between Myocarditis and Synovitis

It has been stated that in scarlet fever there is a connection between the joint and myocardial changes which most frequently are manifested as A-V block (Beer 1938, Holz 1939, Roelsen 1941). The present study would seem to support this conclusion. In the group of 110 cases of myocarditis there were 11 with synovitis or arthralgia, corresponding to 10 per cent; while in the other 2,721 patients there were only 35, or 1.3 per cent, with synovitis. The difference, 8.7 ± 2.9 per cent, is significant, and it may be concluded that synovitis develops more readily in patients who get myocardial damage than others. Of the 11 synovitis cases above, 7 had A-V block. Two of these also had alterations of the T wave, while the remaining 4 showed T wave changes only. It is noteworthy that while changes in the T waves predominated in the remainder of the material, A-V block was the commonest alteration among those with synovitis. The relationship between myocarditis and synovitis cannot depend entirely on the presence of the streptococcal infection and thus it is related to some other factor such as the severity of the primary infection or the reaction of the organism to this.

The Influence of the Severity of the Scarlet Fever

It might be expected that the development of a scarlatina myocarditis would depend more on the mere presence of the toxin rather than on its amount (and thus the severity of the illness) if an allergic factor is of significance in the development of the myocardial lesion. On the other hand, it must be anticipated that in certain cases longer exposure and larger quantities of the toxin are required to bring out this type of reaction. In any case it must be possible for the severity of the reaction produced to vary according to the quantity of the antigen. Roelsen (1941) and others also report a higher incidence of myocarditis if the onset is more severe and accompanied by general systemic involvement and high fever although this relationship has not been statistically analyzed.

The Influence of Age

The higher incidence of myocarditis in the adults than in the children in this study is in agreement with earlier experience. It has been demonstrated, although without statistical analysis, that the incidence of myocarditis increases in the older age groups (Wickström 1932, Granrud 1949, Steinmann 1945); and Spang

and Welsch (1947) have pointed out that the myocardial lesion in older subjects tends to be more serious.

The worsened prognosis associated with a history of previous infections has already been mentioned. The higher incidence of myocarditis and the more severe alterations in the older subjects may possibly depend on the greater likelihood of earlier infectious diseases and development of allergic tendencies in such patients. It must also be asked whether or not the generally more severe illness seen in adults may be of importance.

It is hardly necessary to discuss the generally recognized fact that scarlatina, as well as many of the other so-called children's diseases, follow a more severe course in adults. As an illustration it may be mentioned that among the adults in this study there was a larger total number of severe cases of scarlatina with signs of general toxicity than in the much larger group of children.

The antistreptolysin titer is of considerable interest in this connection. It reflects only one side of the process of antibody formation but probably can be considered as representative. A more persistent initial pyrexia with increased toxin formation results in a higher antistreptolysin titer as Lagercrantz (1950) has demonstrated on a large group of adults and children. Thus the value of the titer would seem to be in proportion to the severity of the illness. Lagercrantz also showed that it is significantly lower in adults than in children. This implies that the antistreptolysin titer production of adults is generally lower than that of children since despite the generally more severe course they developed lower titers. Consequently it is not possible to demonstrate any direct correlation between the titer and the incidence of myocarditis for the entire material. The question is whether this decreased capacity for antibody response may not be one of the factors which contribute to the higher myocarditis morbidity of the adults.

Influence of Penicillin Treatment

Earlier experience

Treatment with penicillin results in a shortening of the course of the primary illness, a shortening of the febrile period, an obvious decrease in the sedimentation rate, disappearance or marked decrease in the hemolytic streptococci and of the associated bacterial complications, and a decrease in the antistreptolysin titer. These facts have been generally documented by a great number of studies. With regard to the duration of the eruption, however, the majority of authors have been unable to find any change (Esser 1949, Galperin 1948, Hoen and Kaiser 1949, Jersild 1947, Stammbach and Larcher 1948) although Hoyne and Brown (1947) found a decrease.

Of greatest interest here is the situation with regard to late toxic complications — nephritis and myocarditis. In a number of studies in which penicillin was used these complications were not observed, but as concurrent control cases

were not studied it is impossible to determine whether this was due to the character of the infection or the treatment. A number of authors, however, have observed nephritis (Galperin) and myocarditis (Bengtsson et al. 1951, Esser 1949, Granrud 1949, Hottinger 1949—50, De Linde and Nielsen 1948, Ondrejicka 1949, Schmeiser and Mattheck 1950, Weinstein et al. 1950) despite the use of penicillin. In Jersild's extensive studies in Copenhagen myocarditis was relatively uncommon, and it was the only complication which did not decrease in a group treated with penicillin as compared with the one treated with sulfa drugs.

Of those authors who have reported a concurrent control series it would seem that Behr (1950) was the only one who has found a decreased incidence of myocardial involvement in the group treated with penicillin. Herrlich (1949) reports an unaltered incidence of nephritis and rheumatic involvement and Lasch (1950) and Paetzold (1950) of myocarditis. There was no detailed analysis of the electrocardiographic changes in these studies, however.

Experience in this study

The most striking result of penicillin treatment was the decrease in the incidence of "*severe*" electrocardiographic changes in adults. There was also a tendency towards a decrease in the incidence of myocarditis in general in adults. The same tendency was present in the children although it was not so convincing since the difference did not come up to a statistically significant value despite the large series.

Can these practical observations be explained theoretically? The presence of an allergic factor in the pathogenesis of myocarditis would seem to explain satisfactorily that the penicillin treatment did not have any great effect on the incidence of myocarditis. The mere presence of the toxin, even if of short duration, would thus be sufficient to set the myocarditis mechanism in motion, and thus a shortening of the course of the primary illness could not prevent the development of a myocarditis. The observed tendency to a decrease may depend on the protection of a small number of subjects who require longer exposure and exposure to a larger quantity of antigen to produce a reaction. This factor would obviously be most important in older subjects who have more severe illnesses and in whom treatment with penicillin would have a greater effect on the formation of the toxin. The relative incidences also indicate that in the material studied the number of subjects who were theoretically threatened with myocarditis but who could be protected with penicillin was small among the children but somewhat larger in the adults. Recent epidemics of scarlet fever have been rather mild in character. When treatment was instituted, which usually took place on the third to fourth day of illness, the patient was often on his way to recovery and children especially practically afebrile. The primary production of toxin was then probably not very marked affected. It seems likely that should the type of infection become more serious the "penicillin effect"

seen in adults might be more pronounced and appear in corresponding fashion in children.

Penicillin's effectiveness in reducing the incidence of "severe" electrocardiographic changes in adults would seem to be of theoretical as well as practical interest. A conceivable interpretation might be that actually it was not possible to prevent the antigen-antibody reaction but that the lesion was of lesser extent due to a reduction in the quantity of antigen.

The possibility of the toxin's having a directly damaging effect cannot be ruled out, of course; but if this was the case an effect more directly correlated with the decrease in the amount of toxin would be expected. A complicated antigen-antibody mechanism would seem to explain the observations of the present study and the clinical characteristics of late scarlatina myocarditis more satisfactorily.

The present observations as to the effect of penicillin seem to agree well in principal with those reported by Weinstein et al. (1950). These workers studied the incidence of rheumatic fever and glomerulonephritis with extraordinary accuracy and careful criteria in 294 cases of penicillin-treated scarlatina of whom half were adults. Late electrocardiographic changes were of considerable importance in making the diagnosis of rheumatic fever which was made in 7 per cent of the cases. The authors point out that the incidence of rheumatic manifestations is relatively high despite penicillin but that strikingly enough the course was so extremely mild that the changes would probably not have been noticed except for the careful examinations.

Conclusion

In view of the present findings regarding the incidence of myocarditis, early penicillin treatment should be instituted in *adults*. With regard to myocarditis in *children*, it would seem that with infections of the current mild type treatment may be omitted or delayed without significant disadvantages. This is of practical importance in regard to the disquieting tendency to development of recurrences following early treatment of scarlatina with penicillin.

CHAPTER 17

Symptomatology and Prognosis

Symptomatology

In this study chief emphasis has been placed on the electrocardiographic changes. In the material as a whole the symptomatology of myocarditis has not been considered consistantly, since examination and progress notes have been made at irregular intervals and by different members of the staff in addition to the author. Because of the practical importance of these circumstances it would seem to be desirable to illuminate them with a short summary of our experience, however.

A. Signs

1. Evidence of decompensation

Ordinary signs of cardiac decompensation — edema, cyanosis, dyspnea, and engorgement of the liver — were observed in only one case. This patient (record no. 883/49, page 123) revealed transient cyanosis and liver engorgement.

2. Auscultatory findings

In this study, as in the literature in general (Wickström, 1931, Steinmann, 1945), no connection between murmurs and myocarditis has been evident. One patient with myocarditis was found at follow-up examination to have a systolic murmur of organic nature which was clearly audible even in the axilla.

This was a 15 year old boy (record no. 4289/47, page 161) who had grade I A-V block both during the scarlet fever as well as earlier with tonsillitis. X-ray of the heart showed that a marked enlargement of the heart had taken place since the scarlet fever confinement three years earlier and that the heart volume was now 520 cc./M.^{2} of body surface. It would thus seem possible that an endocarditis also was present in this case, although nothing worthy of note was found on auscultation during the course of the illness.

In still another myocarditis patient a previously undiagnosed organic heart disease was discovered at the follow-up examination and is now being studied. This seems likely to be a congenital lesion. (Record no. 1981/47, page 129). In one case a gallop rhythm during an active myocarditis suggested the diagnosis. In another there were obvious changes in the strength of the first tone during a transitory period of grade III A-V block. This patient had a coincident fall in blood pressure.

3. The relationship between electrocardiographic changes and the sedimentation rate and temperature

Since the ordinary physical examination of the patient seldom provides evidence of myocarditis, it may be asked whether the temperature or the sedimentation rate are of value.

The majority of the 110 myocarditis patients displayed an increase in the sedimentation rate or microsedimentation rate (Ström, 1933), although the test was normal in 32 cases. Of the latter, 3 had temperature elevations, and 8 had symptoms of heart involvement.

Fever was present at the onset of the myocarditis in only 23 cases, while 22 patients had coincident complications. Five had synovitis, 1 nephritis, and several had cervical lymphadenitis. In the remainder there were upper respiratory infections — pharyngitis, rhinitis, sinusitis, and otitis. As a rule it was also possible again to culture hemolytic streptococci.

Roelsen (1941) and Weinstein et al. (1950) have also observed that both the temperature and the sedimentation rate may have become normal at the time of onset of myocarditis.

B. Symptoms

A question of greatest practical importance is of course the extent of actual "heart trouble" present in these cases of myocarditis diagnosed on the basis of changes in the electrocardiogram. Should it be found even after going into the history carefully that a myocarditis has produced neither signs nor symptoms there is a questionable value in making the diagnosis. This, however, is not the case. Thirty of the 110 patients, or thus somewhat more than one-fourth, have experienced subjective discomfort from their myocarditis despite the fact that a considerable amount of difficulties has probably been avoided by keeping the patient quiet. It must be emphasized that the signs and symptoms are markedly dependent on the load placed on the heart. In many cases the first difficulty has been noticed at the time the patient has begun to resume his normal physical activities some time after regression of the electrocardiographic changes. The actual figures for the symptoms are minimum values, however, since the progress notes were written by different doctors and the follow-up examinations were made so long after the illness that many patients had probably forgotten earlier difficulties.

Of the patients confined to bed in the hospital only 2 experienced symptoms; and in these two women the changes were severe — total A-V block and grade II A-V block — and both complained of precordial pain. One woman (record no. 921/47, page 128) was free of symptoms in the hospital although her electrocardiogram showed isoelectric T waves. She left on a trip during the convalescent period and experienced a severe attack of angina pectoris while travelling. She was admitted to a local hospital where she was obliged to remain for 6 months.

One 8 year old girl (record no. 4162/47, page 93), with a recurrent conduction disturbance, experienced a heart attack several months later one evening after she had gone to bed. She sat up in bed, gasped for breath, tore at her breast, and cried out that she couldn't get air. Fortunately, it is seldom that such dramatic difficulty develops, but many myocarditis patients are afflicted with shortness of breath, fatigue, or anginal discomfort for months and sometimes even longer after the electrocardiogram has become normal.

Dyspnea was the commonest symptom, being experienced by 14 of the patients. *Precordial pain* has been reported by 10 patients. Five of them had typical angina usually occurring on exertion but also experienced in the form of nocturnal pain. The others have reported tingling or aching over the precordium. Four patients have complained of palpitations.

A marked general fatigue has been noted by the patients as frequently as dyspnea, and some of the patients have had psychic trouble. However, since these symptoms are entirely non-specific they have not been counted as subjective heart trouble. A certain type of fatigue seems to be more significant and was spontaneously reported by six mothers whose children had myocarditis. They reported that during the first period following the illness the children have quite spontaneously lain down on the ground while at play or gone in to bed.

In this connection it should be pointed out that in all likelihood myocarditis sometimes occurs without producing changes in the electrocardiogram and that in other cases the electrocardiographic changes are so transient that they evade discovery. In these patients a diagnosis of myocarditis may of course be indicated by the development of clinical signs or symptoms. In a few cases the clinical record has revealed that subjective difficulties referable to the heart have developed subsequent to the illness in the presence of an entirely normal electrocardiogram. (Record nos. 571/47, page 101, and 2572/49.) Follow-up examination with a review of the history has strengthened the impression that a transitory cardiac lesion has been present. These cases have not been counted among those with myocarditis, however, since this diagnosis in the present study is based on changes in the electrocardiogram.

In conclusion it may be stated that one-fourth of the patients with myocarditis have reported subjective discomfort referable to the heart. As a rule these have been moderate or mild but have persisted for a longer time than the electrocardiographic changes.

Prognosis

In the follow-up examinations made from 1 to 5 years following the illness an attempt has been made to gain a conception of the prognosis for the immediate years following the illness. These examinations have been discussed several times previously, and at this point only a short summary of the results with regard to the 110 patients with myocarditis is offered. Ninety-six of the patients have submitted to this follow-up examination. In addition to "in rest" the

electrocardiogram has frequently been recorded after work in order to bring out possibly latent lesions. In many cases, especially if alterations of a more severe type were present, actual function tests have also been made. In addition the patient has been questioned with regard to subjective difficulties referable to the heart and his functional capacity following the myocarditis. A careful auscultation of the heart has been made and the blood pressure checked.

As mentioned in previous chapters the rest electrocardiogram was normal at the time of the follow-up examination in all but 6 patients.

One of these exceptions was the previously mentioned woman with coronary insufficiency (record no. 921/47, page 128). This patient also suffered with hypothyroidism however, and this was probably a factor both in the etiology and prognosis. The others had prolonged conduction times. One of these (record no. 619/49, page 93) happened to be acutely ill at the time of the follow-up and had a recurrent A-V block, but his electrocardiogram was normal again after a short time. The other 4 patients, one woman (record no. 2496/47, page 90) and 3 children (record nos. 4162/47, 1776/49, and 3653/48), seem to have developed disturbances of a more persistent nature. The woman had a grade II A-V block for nearly a year, and it was found to have recurred 4 years later at which time the patient was rather much of an invalid. The children were too young to make a function test, but after work the conduction times were shortened although not always to a normal value. These patients also seem to differ from other children by having increased shortness of breath, decreased endurance, and sudden fatigue on physical exertion. According to a message from a local hospital a seventh patient (record no. 2097/48) had been admitted with rheumatic fever (probably also an endocarditis) at the time that she was supposed to be seen for follow-up examination. An electrocardiogram sent from this hospital showed flattening of the T wave similar to that during the scarlet fever.

Three patients showed changes in the work electrocardiogram. One patient who had had a grade I A-V block was found to have a latent conduction disturbance. This was a 13 year old boy (record no. 3309, page 92) whose rest electrocardiogram was normal with a P-Q time of 0.16 seconds. He was able to manage a function test of 750 Kg.M./min. satisfactorily, but afterwards the P-Q time at a pulse of 110 was prolonged to 0.24 seconds, a pathological work reaction. A year later the P-Q time was found to be 0.30 seconds in rest, but the functional capacity was entirely normal — 1200 Kg.M./min., and following the test the P-Q time was shortened to 0.23 seconds. An x-ray of the heart showed a volume of 410 ml./M.^{2} of body surface, and the contour of the right auricle was somewhat larger than normal.

The other case was that of a man (record no. 3616/47, page 70) who displayed ventricular extrasystoles in the work electrocardiogram although the rest electrocardiogram and function test were normal. The earlier electrocardiographic changes in this case, however, were negative T waves, and it is questionable whether the scarlet fever could have given rise to these premature beats occurring several years later. In still another patient (record no. 669/49, page 47) a previously observed coronary sinus rhythm of active type reappeared in the work electrocardiogram.

In all the rest of the patients who were tested both the function test and the work electrocardiogram were normal. However, two women stated that they did not feel that they had fully returned to former physical condition although their functional capacity was within normal limits. All other patients had become free of symptoms.

Attention has been given to *murmurs* as a possible indication of endocarditis caused by scarlet fever. In one patient (record no. 4289/47, page 182) valvular

heart disease had developed. Obviously this period of observation is entirely too short to permit any definite conclusions about valvular heart disease due to scarlet fever. Some suspicious cases have been examined with the phonocardiograph which in none of these cases has indicated that the murmur was of organic origin.

In conclusion it may be stated that the follow-up examinations have confirmed the impression that scarlatina myocarditis is of a benign character with complete recovery according to both the electrocardiogram and the function test in the majority of cases. Auriculoventricular conduction disturbances, especially those with early onset, seem, however, to have a more serious prognostic implication than other types of disturbances judging from the results of this study.

Summary

The *purpose* of this work has been to study the effect of penicillin on myocarditis associated with scarlet fever. This has been done by means of electrocardiographic examinations on concurrent groups of patients with scarlet fever treated both with and without penicillin. To accomplish this it has been necessary to make a thorough study of the electrocardiograms in order to differentiate between alterations resulting from acute myocarditis and others caused by physiologically normal variations, constitutional anomalies, or old lesions.

The *material* (table I) consists of 450 cases of scarlatina treated with penicillin, 440 controls who received no penicillin, and a concurrent group of 211 in whom the initiation of penicillin was delayed until the fifth hospital day. In addition there are two later treatment groups consisting of 707 patients altogether with an incomplete control group of 99. These cases as well as a number in the desquamating stage and others with scarlatina from the same period have also been included since they are of interest with regard to the electrocardiographic studies. Altogether the material includes 2,831 patients of whom 279 were adults.

Observed General Principles in Evaluating the Material

1. Electrocardiograms were recorded serially and each series evaluated individually.

2. Even in patients whose hearts are not affected the electrocardiogram can be expected to vary more than ordinarily during an infectious illness with its different phases of sympathetic and vagus influence.

3. The normal electrocardiogram of the individual can be established after recovery (as a rule the myocarditis of scarlet fever is reversible). Stimulation of the sympathetic and vagus factors, "work"-electrocardiograms, and recordings of the electrocardiogram in various body positions have been employed in this connection to study the normal variability in different subjects.

4. A pathological alteration in the electrocardiogram can be considered to justify a diagnosis of myocarditis only if an association with the scarlatina can be demonstrated. On the other hand a variation within the accepted normal limits may be an indication of myocarditis if it lies beyond the normal limits of variability for the subject in question.

5. Alterations apparently within physiological limits may also be due to a myocarditis. If, in addition, subjective cardiac complaints have been reported the patient has been included in a special group designated by "probable myocarditis with equivocal electrocardiographic changes and heart symptoms".

Electrocardiographic Findings

A survey of the electrocardiographic changes is given in table 8.

Alterations of the P wave have been analyzed. The majority of these were apparently due to minor changes in the position of the pacemaker (called "perisinus rhythms" by the author) and are physiologically normal in character. A definite intra-auricular conduction disturbance was present in only one case.

Coronary sinus rhythm or atrioventricular nodal rhythm have been observed in 43 cases (table 2). These have been analyzed with reference to whether they showed signs of being active or passive and whether they were associated with the scarlet fever. In these cases 8 were definitely active and have been interpreted as evidence of myocarditis, while 3 were of questionable nature. The others give impression of having been passive physiological wanderings of the pacemaker associated with decreasing sinus frequency or increased vagus effect.

Premature systoles have been observed in 31 patients (table 3). Although 5 of these were pathological in character only 1 of them displayed a definite association with a scarlatina myocarditis.

Parasystoles of the ventricular type have been diagnosed in 5 patients, and in 1 of them the cause seems to have been a myocarditis.

Escaped beats of nodal origin have been observed in 9 patients. Ventricular escaped beats were seen in 2 cases both of whom have been considered to have myocarditis.

Auriculoventricular conduction disturbances have been regarded as resulting from myocarditis in 34 patients. Among these were 1 case of total block and 2 of grade II block (table 5). Four of the subjects with myocarditis had P-Q values within accepted normal limits, but the range of variation and the overall series of electrocardiograms in these cases indicated a temporary disturbance during the illness. Another 26 patients were found to have prolonged P-Q intervals; and 12, large variations in the P-Q time, although these remained unaltered over a period of one or several years. In 3 different families two or three siblings displayed prolonged P-Q times, and a constitutional anomaly is regarded as the probable explanation.

Changes in the T wave and the S-T segment. Changes in the T wave of various degrees (table 6) which have been interpreted as evidence of a scarlatina myocarditis have been observed in 57 cases, and depressions of the S-T segment with the same significance have been found in 11 patients. In another large group of patients the alterations were found to have a possible connection with sympatheticotonic factors or changes in position.

Intraventricular conduction disturbances have been observed in 29 patients; but in only 2 cases, both of whom displayed an intermittent Wilson block, would a scarlatina myocarditis seem to have been the explanation. The remaining cases displayed no change during a long period of observation. Two pairs of siblings displayed similar alterations seeming to indicate the presence of a constitutional anomaly. Pre-excitation was found in 7 cases although only 1 appeared to be related to the scarlet fever.

The Results with Regard to the Incidence of Myocarditis

The series are summarized in tables 9 to 11. Certain cases of myocarditis with more marked changes in the electrocardiogram have been designated as "severe".

The effect of the frequency with which the electrocardiograms are recorded is demonstrated by the fact that the incidence of myocarditis diagnosed in a group of 208 patients rose from 3.9 per cent when the electrocardiogram was recorded once a week to 5.8 per cent when 3 recordings were made each week.

The *age factor* is illustrated by the significantly higher total incidence of myocarditis in adults than in children (11.5 per cent, as compared with 3.1 per cent) as well as the more "severe" electrocardiographic changes (5.0 per cent compared to 0.24 per cent).

Seasonal variations during a year are seen in figure 46 which shows that the children have a probably higher incidence of myocarditis during the winter season compared with the summer. The same tendency was displayed by the adults.

A comparison between the cases receiving penicillin and the controls reveals that all groups of adults and all but one group of children receiving penicillin early in the illness, had a lower incidence of myocarditis than the control groups.

In the *adults* a statistical analysis of the individual groups revealed no significant difference. When 2 penicillin groups and their controls were combined the difference in the incidence of myocarditis between the penicillin cases and the controls rose to 11.8 ± 5.5 per cent. With regard to "severe" alterations the difference was probable -9.4 ± 3.8 per cent.

Patients who began to receive penicillin later in the illness reveal a higher incidence of myocarditis than those receiving early treatment but a lower one than the controls. The incidence of "severe" electrocardiographic changes in all of the adults receiving early penicillin treatment in the entire study is 0.84 per cent, while in all other adults: controls, desquamators, and those receiving late penicillin treatment, the incidence was 8.8 per cent. This difference is statistically significant.

In *children* the differences do not come up to a statistically definite value.

The *desquamating cases* display a lower observed incidence of myocarditis than the control cases. This is probably due to the limited period of observation

for most of the desquamators. In a group of 107 patients in which the scarlet fever was complicated by the presence of *other infectious diseases* no definite difference as compared with the rest of the material could be established.

Summary: Penicillin is apparently able to reduce the incidence of "severe" electrocardiographic changes in scarlet fever although it shows only a tendency to reduce the actual incidence of myocarditis in association with the disease.

Of course these observations and conclusions are valid only in milder epidemics of the sort seen in recent years. Should a more severe type appear, penicillin therapy would in all likelihood assume a greater importance with regard to myocarditis in both adults and children.

Conclusion

In view of the present findings regarding the incidence of myocarditis, early penicillin treatment should be instituted in *adults*. With regard to myocarditis in *children*, it would seem that with infections of the current mild type treatment may be omitted or delayed. This is of practical importance in regard to the tendency to development of recurrences following early treatment of scarlatina with penicillin.

Statistical Formulas and Abbreviations

Number of patients observed = n.
Number of patients who developed myocarditis = f.
The standard error = ε.

The standard error of a percentage, $p = \frac{f \times 100}{n}$, is calculated according to the following formula:

$$\varepsilon(p) = \sqrt{\frac{p(100-p)}{n-1}}$$

The standard error of a difference between two percentages, p_1 and p_2, is calculated according to the formula:

$$\varepsilon(p_1-p_2) = \sqrt{\varepsilon(p_1)^2+\varepsilon(p_2)^2}.$$

Definition of significance and probability of a difference, $D \pm \varepsilon(D)$:
Significance: $D \geqq 3\varepsilon(D)$.
Probability: $3\varepsilon(D) > D \geqq 2{,}5\varepsilon(D)$.

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Resume

Le sujet de ce travail a été d'étudier l'effet de la pénicilline sur les myocardites associées à la scarlatine. Ceci a été fait au moyen d'examens électrocardiographiques sur des groupes comparables de malades atteints de scarlatine et traités avec ou sans pénicilline. Pour cela, il a été nécessaire de faire une étude complète des électrocardiogrammes afin de différencier les altérations résultant d'une myocardite aigüe de celles causées par les variations physiologiques normales et de celles provenant d'anomalies constitutionnelles ou de lésions anciennes.

Cette étude porte sur 450 cas de scarlatines (tableau I) traités par la pénicilline, 440 témoins qui n'ont pas reçu de pénicilline et un groupe comparable de 211 sujets chez lesquels le début de la pénicillinothérapie a été retardé jusqu'au cinquième jour de l'hospitalisation. En plus, il y a deux groupes classés selon la mode de traitement pénicilliné et comprenant 707 malades avec un groupe témoin incomplet de 99 sujets. Ces cas aussi bien que certains vus au stade de desquamation et d'autres vus avec une scarlatine datant de la même période ont également été inclus dans cette étude puisqu' ils présentent un intérêt du point de vue des études éelctrocardiographiques. Au total l'étude comprend 2831 sujets dont 279 adultes.

Principes généraux observés dans le choix des cas étudiés

1. Les électrocardiogrammes ont été enregistrés en séries et chaque série appréciée individuellement.
2. Même chez les sujets dont le coeur n'a pas été touché, l'électrocardiogramme peut varier plus qu'à l'ordinaire pendant une maladie infectieuse selon les différentes phases d'influence sympatique et vaguale.
3. L' électrocardiogramme normal d'un individu peut être établi après la guérison (puisque en règle la myocardite de la scarlatine est réversible). La stimulation des facteurs sympatiques et vagaux, les "work" électrocardiogrammes et les résultats des examens selon les différentes positions du corps ont été utilisés dans ce but.

4. On peut retenir une altération pathologique de l'électrocardiogramme pour justifier le diagnostic de myocardite seulement si l'on peut démontrer l'association avec la scarlatine. D'autre part, une variation entre les limites normales habituelles peut être une indication de myocardite si elle se trouve au delà des limites de variabilité du sujet en question.

5. Des altérations apparemment dans les limites physiologiques peuvent aussi être dues à une myocardite. Si, de plus, des troubles cardiaques subjectifs ont été rapportés, le malade a été classé dans un groupe spécial appelé "myocardite probable avec altérations électrocardiographiques douteuses et symptômes cardiaques".

Résultats électrocardiographiques

Les altérations de l'onde P ont été analysées. La majorité étaient apparemment dues à des changements infimes de la position du pacemaker (appelé "perisinus rythms" par l'auteur) et sont de caractère physiologiquement normal. Il n'y eut que dans un seul cas un trouble défini de la conduction intra-auriculaire.

Des rythmes sinusaux coronaires et des rythmes nodaux atrio-ventriculaires ont été observés dans 43 cas (tableau 2). Ils ont été analysés soit par rapport aux signes d'activité ou d'inaction qu'ils mettaient en évidence, soit par rapport à leur association à la scarlatine. Parmi ces cas 8 furent actifs en fin de compte et ont été interprêtés comme myocardites évidentes tandis que 3 étaient de nature discutable. Les autres altérations du tracé donnent l'impression d'être causées par une inactivité physiologique du pacemaker associée à la diminution de la fréquence sinusale ou à l'augmentation de l'effet du vague.

Des systoles prématurées ont été observées chez 31 patients (tableau 3). Bien que 5 d'entre eux aient été de caractère pathologique seulement 1 présentait une association certaine avec une myocardite scarlatineuse.

Des parasystoles de type ventriculaire ont été diagnostiquées chez 5 malades, et dans 1 cas la cause semble avoir été une myocardite.

Des "escaped beats" d'origine nodale ont été observés chez 9 malades. Des "escaped beats" ventriculaires furent vus dans deux cas qui tous deux avaient été considérés porteurs de myocardite.

Des troubles de la conduction auriculo-ventriculaire ont été considérés comme résultant d'une myocardite chez 34 malades. Parmi eux était 1 cas de blocage total et 2 de blocage de degré II (tableau 5). Quatre de ces sujets porteurs de myocardite avaient des valeurs P-Q entre les limites habituelles normales, mais l'entendue de la variation et sa présence sur toute les séries d'électrocardiogrammes indiquaient dans ces cas un trouble temporaire au cours de la maladie. On a trouvé 26 autres malades qui avaient un intervalle P-Q allongé, et 12 de

grandes variations dans le temps de P-Q bien que ceux-ci fussent rester inaltéré sur une période d'une ou plusieurs années. Dans 3 familles différentes, 2 ou 3 parents montrèrent un temps P-Q allongé et l'on considère qu'une anomalie constitutionnelle en est l'explication probable.

Modifications de l'onde T et du segment S-T. Des modifications de degrés variés de l'onde T (tableau 6) qui ont été interprêtées comme modifications évidentes de myocardite scarlatineuse ont été observées dans 57 cas et des dépressions du segment S-T, de même significations, ont été trouvées chez 11 malades. Dans un autre groupe important de malades on a trouvé que les altérations pouvaient être en relation avec les facteurs sympathiques ou les changements de position.

Des troubles de la conduction intraventriculaire ont été noté chez 29 patients. mais dans 2 cas seulement on trouve un blocage intermittent du type Wilson, il semblerait que la scarlatine en soit l'explication. Les cas restants ne montrent pas de changements pendant une longue période d'observation. Deux paires de sujets de la même famille montrent des altérations similaires semblant indiquer la présence d'une anomalie constitutionnelle. Une pré-excitation fut trouvée dans 7 cas quoique 1 cas seulement semblait être en relation avec la scarlatine.

Resultats par rapport à l'incidence des myocardites

Les séries sont résummées dans les tableaux 9—11. Certains cas de myocardite avec des changements plus marqués dans l'électrocardiogramme ont été désignés comme "sévère".

L'effet de la fréquence avec laquelle a été enregistré l'électrocardiogramme est démontré par le fait que l'incidence des myocardites diagnostiquées dans un groupe de 208 malades s'élève de 3,9 % quand l'électrocardiogramme a été enregistré une fois par semaine à 5,8 % lorsque trois examens ont été faits chaque semaine.

Le facteur âge a été étudié. L'incidence totale des myocardites est significativement plus élevées chez l'adulte que chez l'enfant (11,5 % contre 3,1 %) aussi bien pour les changements électrocardiographiques plus "sévères" (5,0 % contre 0,24 %).

Les variations saisonières sont indiqués dans la figure 46 qui montre que les enfants ont probablement une incidence de myocardites plus élevée en hiver qu'en été. La même tendance a été retrouvée chez l'adulte.

Une comparaison entre les cas recevant de la pénicilline et les témoins révèle que tous les groupes d'adultes et tous les enfants (sauf un groupe) recevant de la pénicilline tôt dans la maladie ont une incidence plus faible de myocardites que les groupes témoins.

Chez les adultes, une analyse statistique des différents groupes révèle une différence non significative. Quand 2 groupes traités à la pénicilline et leurs témoins furent combinés, la différence dans l'incidence de la myocardite entre les cas pénicillinés et les témoins monte jusqu'à $11{,}8 \pm 5.5\,\%$. En tenant compte des altérations "sévères" la différence était probablement — $9{,}4 \pm 3{,}8\,\%$. Les malades qui reçurent la pénicilline plus tard dans la maladie montrent une incidence plus élevée de myocardites que ceux qui ont reçu un traitement précoce, mais plus faible que celle des témoins. L'incidence des changements électrocardiographiques "sévères" dans tous les cas d'adultes ayant reçu un traitement précoce de pénicilline est de 0.84 % pour l'ensemble de l'étude, tandis que pour tous les autres adultes: témoins, scarlatine à la période desquamative, et ceux ayant reçu un traitement pénicilliné tardif, l'incidence fut de 8,8 %. Cette différence est statistiquement significative.

Chez les enfants les différences n'aboutissent pas à une valeur statistiquement définie.

Les cas à la période desquamative montrent que l'incidence de myocardite est plus basse que pour les cas témoins. Ceci est probablement du à la période d'observation limité de la plupart des cas avec desquamation. Dans un groupe de 107 malades dans lequel la scarlatine a été compliqué d'autres maladies infectieuses on n'a pu établir de différences précises en comparaison avec le reste des cas étudiés.

Résumé: La pénicilline est apparemment capable de réduire l'incidence des modifications "sévères" électrocardiographiques dans la scarlatine bien qu'elle montre seulement une tendance à réduire l'incidence actuelle des myocardites associée à cette affection.

En fait, ces observations et ces conclusions sont valables seulement dans les épidémies plus faibles telles que celles vues ces dernières années. Si une forme plus sévère survenait, la pénicillinothérapie acquérerait selon toute vraisemblance, une importance plus grand vis-à-vis des myocardites à la fois chez l'adulte et chez l'enfant.

Conclusion

Devant les résultats actuels concernant l'incidence des myocardites, un traitement pénicilliné précoce devrait être institué chez l'adulte par rapport aux myocardites, chez l'enfant, il semblerait que dans la forme courante banale, le traitement pourrait être délaissé ou différé. Ceci est d'une importance pratique par rapport à la tendance à l'apparition de récidives après le traitement précoce de la scarlatine par la pénicilline.

Zusammenfassung

Die Absicht dieser Arbeit war es, zu untersuchen, welche Wirkung Penicillin auf Myocarditis hat, die sich bei Fällen von Scharlach zeigte. Es wurde deshalb EKGs von Scharlachkranken aufgenommen. Eine Gegenüberstellung der EKGs sonst gleichartiger Gruppen von Scharlachfällen, von denen jedoch nur jeweils die eine Gruppe Penicillin erhielt, sollte diese Wirkung von Penicillin zeigen. Zunächst musste bei den in Frage kommenden EKGs festgestellt werden, ob die vorhandenen EKG — Anomalien tatsächlich von einer akuten Myocarditis herrührten, oder ob sie auf normale physiologische Ursachen, auf constitutionelle Anomalien, oder auf frühere Schädigungen zurückzuführen waren.

Das *Krankenmaterial* setzte sich zusammen aus: (Tab. 1) 450 Scharlachfällen, die mit Penicillin behandelt wurden; zum Vergleich aus 440 Fällen, die kein Penicillin erhielten und aus einer Gruppe von 211 Patienten, bei denen die Penicillinbehandlung erst am 5. klinischen Tag begonnen wurde. Ferner aus 2 Gruppen von zusammen 707 behandelten Patienten und einer unvollständigen Kontrollgruppe von 99. Ausserdem wurden für diese Untersuchungen eine Anzahl von Scharlachfällen im Stadium der Abschuppung der Haut herangezogen. Das Krankenmaterial umfasst zusammen 2831 Patienten, davon waren 279 Erwachsene.

Allgemeine Gesichtspunkte zur Bewertung der EKGs

1. Von jedem Patienten wurden EKG-Reihen aufgenommen. Die EKGs wurden nach den Besonderheiten jedes einzelnen Patienten bewertet.
2. Es konnte angenommen werden, dass während einer Infektionskrankheit mit ihren verschiedenen Phasen sympatischen und parasympatischen Einflusses das EKG eines Patienten mit einem gesunden Herzen mehr als gewöhnlich variiert.
3. In der Regel ist eine Scharlachmyocarditis reversibel. Die gesunden EKG-Variationen wurden deshalb nach der Genesung des Patienten untersucht. Dabei wurden sympathische und parasympathische Reizmittel, Arbeits-EKG und das EKG in verschiedenen Körperlagen angewandt.

4. Eine pathologische Veränderung des EKG konnte nur dann zur Diagnose Myocarditis berechtigen, wenn der Zusammenhang mit dem Scharlach gezeigt werden konnte. Andererseits waren nur geringfügige EKG-Veränderungen dann ein Zeichen für Myocarditis, wenn sie die Variabilitätsgrenze des fraglichen Patienten überschritten.

5. Veränderungen die offensichtlich in physiologischen Grenzen lagen, konnten ebenfalls auf Myocarditis zurückgeführt werden. Patienten mit zusätzlichen Herz-Beschwerden wurden in eine besondere Gruppe eingereiht mit der Bezeichnung: "Unklare EKG-Veränderungen und Herzsymptome, Myocarditis wahrscheinlich".

Electrocardiographische Ergebnisse

Veränderungen der P Zacke.

Die meisten derartigen Abweichungen waren physiologischer Natur und offensichtlich durch kleine Lageveränderungen des "Schrittmachers" verursacht. (Bezeichnung des Autors: Perisinus Rhythmus) Eine tatsächliche intraaurikuläre Reizleitungsstörung zeigte sich nur in einem Fall.

"Sinus-Coronarius-Rhythmus" oder Atrioventrikulärer Knotenrhythmus wurde bei 43 Fällen beobachtet, aktive und passive Veränderungen unterschieden und die Beziehung zu der Scharlacherkrankung untersucht. Davon waren 3 aktiv als Zeichen einer Myocarditis, 3 Fälle waren fraglich. Die anderen waren allem Anschein nach zurückzuführen auf physiologische Verlagerungen des "Schrittmachers" bei verminderter Sinusfrequenz und erhöhter Vaguswirkung.

Extra-Systolen ("premature systoles") wurden bei 31 Fällen festgestellt (Tab. 3). Obwohl 5 davon pathologischer Natur waren, war nur in einem Fall ein tatsächlicher Zusammenhang mit der Scharlacherkrankung vorhanden.

Parasystolische Kammerarhythmien wurden bei 5 Patienten diagnostiziert und bei einem schien die Ursache Myocarditis zu sein.

Ersatzsystolen ("escaped beats") vom AV-Knoten ausgehend wurden bei 9 Patienten beobachtet. Ersatzschläge, die in tiefer gelegenen Zentren ihren Ursprung hatten, wurden bei 2 Fällen festgestellt, beide hatten augenscheinlich Myocarditis.

Störung der atrioventrikulären Reizleitung als Folge von Myocarditis wurde bei 34 Patienten beobachtet. Darunter war ein totaler Block und 2 Fälle von partiellem Block (Tab. 5). 4 der Fälle mit Myocarditis hatten zwar P-Q-Werte in normalen Grenzen, aber der Umfang der Variationen in den EKG-Reihen bei diesen Fällen zeigten eine vorübergehende Leitungsstörung während der Krankheit. Bei 26 anderen Patienten wurden verlängerte P-Q-Werte gefunden, bei 12 Fällen traten erhebliche Veränderungen in den Ueberleitungszeiten auf.

die allerdings mehrere Jahre unverändert bestehen blieben. In 3 verschiedenen Familien hatten 2 oder 3 Geschwistern verlängerte P-Q Zeiten, vermutlich verursacht durch konstitutionelle Anomalien.

Veränderungen des T und des ST.

Verschiedene grosse Veränderungen der T Zacke (Tab. 6), die als Folgeerscheinung von Myocarditis erklärt wurden, zeigten sich bei 57 Fällen. Eine Senkung des ST-Abschnittes, die augenscheinlich auch auf Myocarditis zurückzuführen war, wurde bei 11 Patienten gefunden. Bei einer anderen grossen Gruppe von Patienten stellte sich heraus, dass die Veränderungen durch sympathikotonische Einwirkungen oder durch Veränderungen der Körperlage verursacht werden konnten.

Eine intraventrikuläre Reizleitungsstörung wurde bei 29 Patienten beobachtet, aber nur bei 2 Fällen, die einen zeitweiligen Schenkelblock vom Wilsontyp hatten, scheint Scharlach die Ursache gewesen zu sein. Die übrigen Fälle zeigten während einer langen Beobachtungszeit keine Veränderungen. Bei je 2 Geschwistern ergaben sich ähnliche Abweichungen, was auf konstitutionelle Anomalien hinwies. Ein WPW-Syndrom (pre-excitation) wurde bei 7 Fällen gefunden, obgleich offenbar nur bei einem Fall eine Beziehung zu der Scharlacherkrankung bestand.

Ergebnisse im Hinblick auf die Ursachen für die Häufigkeit von Myocarditis

Alle untersuchten Fälle wurden in Tabelle 9—11 gruppenweise zusammengestellt. Bestimmte Fälle von Myocarditis mit besonders hervortretenden EKG-Veränderungen wurden als "schwer" bezeichnet.

Die Wichtigkeit einer häufigen EKG-Schreibung zeigte sich dadurch, dass bei einer Gruppe von 208 Patienten die festgestellten Myocarditisfälle 5,8 % statt 3,9 % betrugen, als das EKG nicht einmal sondern dreimal wöchentlich genommen wurde.

Die Bedeutung des Alters wurde durch das häufigere Vorkommen von Myocarditis bei Erwachsenen gegenüber Kindern gezeigt: 11,5 % gegenüber 3,1 %. Auch traten bei den Erwachsenen mehr "schwere" Fälle auf als bei Kindern (5,0 % zu 0,24 %).

Die jahreszeitlichen Veränderungen wurden in Fig. 46 dargestellt, welche zeigt, dass Erwachsenen und auch Kinder im Winter anfälliger sind für Myocarditis.

Ein Vergleich zwischen den Fällen, die Penicillin erhielten und den Kontrollfällen zeigt, dass bei allen Gruppen von Erwachsenen wie auch von Kindern — eine Gruppe von Kindern ausgenommen — die im Frühstadium der Krank-

heit mit Penicillin behandelt wurden, weniger Fälle von Myocarditis auftraten als bei den Kontrollgruppen.

Bei den *Erwachsenen* zeigte sich zwischen den einzelnen entsprechenden Gruppen keine sicheren Unterschiede. Bei einer Zusammenfassung von 2 Gruppen und 2 Kontrollgruppen ergab sich ein Unterschied von 11,8 % ± 5,5 % für die Häufigkeit von Myocarditis. Unter Berücksichtigung der "schweren" Fälle betrug die Differenz 9,4 ± 3,8 %.

Patienten, die zu einem späteren Zeitpunkt der Krankheit Penicillin erhielten zeigten ein häufigeres Auftreten von Myocarditis als jene die früher mit Penicillin behandelt wurden. Die nicht behandelten Kontrollgruppen jedoch zeigten eine noch grössere Häufigkeit als die spät behandelten Fälle. Die Häufigkeit von "schweren" EKG-Veränderungen bei allen erwachsenen Patienten, die für diese Arbeit herangezogen wurden und eine frühe Penicillinbehandlung erhielten, ist 0,84 %, während bei allen anderen erwachsenen Patienten, — Kontrollgruppen, Patienten im Stadium der Hautabschuppung und solche, die erst spät Penicillin bekamen — die Häufigkeit von Myocarditis 8,8 % betrug. Dies ist ein sicherer statistischer Unterschied.

Bei *Kindern* erreicht der Unterschied keinen definitiven statistischen Wert.

Bei den Patienten im Stadium der *Hautabschuppung* wurden weniger häufig Fälle von Myocarditis gefunden als bei den Vergleichfällen. Dies ist möglicherweise darauf zurückzuführen, dass die Beobachtungszeit für diese Patienten kürzer war.

Bei einer Gruppe von 107 Fällen, bei denen eine *Komplikation mit anderen Infektionskrankheiten hinzukam*, konnte im Vergleich zu den Fällen ohne diese Komplikationen keine deutlichen Unterschiede festgestellt werden.

Ergebnis:

Penicillin gibt offensichtlich die Möglichkeit "schwere" EKG-Abweichungen, die bei Scharlach auftreten zu vermindern. Ebenso ist anzunehmen, dass Penicillin die Häufigkeit von Scharlach-Myocarditis vermindert.

Folgerungen:

Zur Verhütung von Myocarditis sollte bei Erwachsenen eine frühe Penicillinbehandlung vorgenommen werden. Bei Kindern kann bei Infektion mit der bekannten leichten Form von Scharlach die Penicillinbehandlung unterlassen oder später begonnen werden. Dies ist von praktischer Bedeutung im Hinblick auf die Rückfälle, die bei einer Scharlach-Frühbehandlung auftreten.

Natürlich haben diese Beobachtungen und Folgerungen nur Gültigkeit für die leichteren Formen dieser Epidemie, wie wir sie in den letzten Jahren hatten. Sollten jedoch schwerere epidemische Formen auftreten, so wird aller Wahrscheinlichkeit nach Penicillin für die Verhütung von Myocarditis eine grössere Bedeutung gewinnen, sowohl in der Behandlung von Erwachsenen als auch von Kindern.

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OTO-RHINOLOGICAL INFECTIONS IN CHILDHOOD

Sero-Bacteriological Studies of Paranasal Sinusitis and Suppurative Otitis with Special Reference to Haemophilus Influenzae

BY

GÖSTA TUNEVALL

Almqvist & Wiksells Boktryckeri AB UPPSALA

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6. **Rhodin, Hans,** The Suspension-Stability of the Blood in Scarlatina. Sw. Cr. 3: —.
7. The Proceedings of the Fourth Scandinavian Pædiatric Congress, held at Helsingfors on June 28th—30th, 1927. Sw. Cr. 5: —.
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From the Municipal Bacteriological Central Laboratory, Stockholm.
(Head Prof. H. Davide, M.D.)

OTO-RHINOLOGICAL INFECTIONS IN CHILDHOOD

Sero-Bacteriological Studies of Paranasal Sinusitis and Suppurative Otitis with Special Reference to Haemophilus Influenzae

BY

GÖSTA TUNEVALL

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Introduction

Since 1949 investigations have been carried out in order to elucidate the occurrence of the common pathogenic bacteria of the upper respiratory tract in children, their pathogenicity, and the immune responses of children to these bacteria.

In order to cover the major part of this field, the studies comprised pneumococci, haemolytic streptococci, influenza bacilli, and pyogenic staphylococci, as well as immune reactions to these bacteria. Special attention was paid to the influenza bacilli as previously often neglected in these connexions. For the study of these bacteria certain bacteriological and serological procedures were developed.

As a basis for the study of infectious processes, "normal" control groups were examined, and the diaplacental transmission of antibodies from mother to child was studied. The infectious conditions were represented by sinusitis and otitis cases in children up to seven years of age. These clinical materials were collected and studied in cooperation with Dr. G. Bjuggren of the Ear, Nose, and Throat Department of the Sabbatsberg Hospital in Stockholm.

The parallel study of the four bacterial species mentioned and the corresponding immune reactions, together with the age distribution of the material, made the results applicable as a basis for a discussion of the characters particular to infections of different aetiology and of the age-conditioned variation of antibody formation, as well as of the joint influence of these factors on the course of the infections in childhood.

The investigations have been published separately. The present report comprises largely a summary and a discussion of the contents of the following papers:

Studies on Haemophilus influenzae

I. Type characteristics. Acta path. & microbiol. Scand. 30:203, 1952.
II. Transfer of capsule formation ability and type specificity to non-capsulated respiratory strains. Ibid. 31:233, 1952.

III. Susceptibility *in vitro* to antibiotics. Ibid. 29:203, 1951.

IV. A complement fixation test for *Haemophilus influenzae* antibody. Ibid. 1952.

V. *Haemophilus influenzae* antigens studied by the gel precipitation method. Ibid. 1952.

Other investigations

VI. The antipneumolysin reaction and its clinical application. Scand. J. Clin. & Lab. Invest. 1952.

VII. Sero-bacteriological investigations of sinusitis in children. Acta otolaryng. 1952.

VIII. Otitis in childhood. A clinical and sero-bacteriological study with special reference to the significance of *Haemophilus influenzae* in relapses. Ibid. 1952.

In the following, references to these papers will be made in Roman figures. Arabic figures refer to the general reference list.

Investigations Underlying the Clinical Study

Studies on Haemophilus Influenzae

Type Characteristics

According to PITTMAN's investigations of 1930 and 1931 (87, 88), the species *Haemophilus influenzae* contains encapsuled forms and non-capsulated ones. The latter, often recovered from the upper respiratory tract of healthy subjects, have also been observed in minor respiratory ailments and their purulent complications (3, 14), and occasionally also in meningitis (47). The non-capsulated, or rough (R), strains form small colonies containing bacilli of various length. The antigenic pattern is complicated and involves as well species-specific factors as group- or even strain-specific antigens of a distribution too irregular to permit a serological classification of the species (88, 40, 23, 89).

The encapsuled, or mucoid (M), form is responsible for severe infections in children, such as meningitis (87), acute septic laryngitis (57), and broncho-pneumonia with empyema (88), conditions which are often associated with bacteraemia. It is virulent for mice (40) and, unlike non-capsulated bacilli, resistant to the bactericidal power of normal serum (116). Encapsuled bacilli have also been found in maxillary sinusitis (3) and suppurative otitis (14), as well as in the nasopharynx of healthy children (3, 95, 30). Encapsuled cultures form large, smooth or mucoid colonies, generally consisting of coccoid bacilli, and on appropriate media iridescent in obliquely transmitted light (87). In addition to their somatic antigens, which do not essentially differ from those of non-capsulated strains, they possess capsular muco-polysaccharides (46, 72) which are precipitable with homologous antisera and decisive for the type specificity. Six types have been recognized and named from a to f (87, 88). Type e presents a subgroup containing an additional capsular antigen (113). Apart from precipitations, type determination may be achieved by capsular swelling reactions (2) or agglutination tests which, however, result in irregular cross-reactions due to interference of somatic antigens, if the incubation time or temperature is increased over certain limits (88, 23). The relations between encapsuled and non-capsulated strains will be more fully discussed in a following section.

Determination of Type in Encapsuled Influenza Bacilli

The "Neufeld technique" was applied by ALEXANDER (2) to influenza bacilli, but does not always yield clear-cut results. It was modified by ENGBAEK (37) who performed capsule swelling with young growing cultures on Levinthal agar. This method was further improved by the author (I) by employing the following procedure.

Two drops of melted Levinthal agar poured on a sterile slide were allowed to solidify as a thin layer. The agar was inoculated with one drop of an 18-hours Fildes broth culture diluted 1:10 and for six hours incubated in a moist chamber at 37 C. One drop of antiserum was added to the culture and, after another half hour of incubation, a loop-ful of methylene-blue solution. A thin cover glass was placed on the culture which was then examined under the microscope. Type-specific rabbit antisera produced by immunizing with fresh vaccines prepared from young cultures in the state of optimum capsule formation were used.

The thin and translucent agar layer permitted sharp pictures. During the incubation after the adding of antiserum, the cells in positive reactions collected to rounded "micro-colonies", with the swollen capsules clearly visible at the margins and sharply contrasting to the scattered organisms of the negative tests, as shown in Figure 1, opposite to page 12.

It has been suggested that *H. influenzae* strains might exist, intermediate between the smooth and the rough forms, and endowed with type specificity but too poorly encapsuled as to display a capsule swelling phenomenon. By phenol extraction traces of capsular antigen should be obtainable from such strains and recognizable by precipitation tests (70). However, attempts along these lines could not extend type specificity to strains not typable by the method already described (I).

Serological Relations to Pneumococci

Serological relations between *H. influenzae* capsular antigens and those of pneumococci have repeatedly been demonstrated and more closely investigated by NETER (79) who found, in anti-*H. influenzae* horse serum, two antibody fractions, one of which reacted with pneumococcal polysaccharide. The corresponding fraction in rabbit antiserum has been found to make up from 1 to 40 % of the antibody content (71).

In capsule swelling tests, the author was able to confirm the existence of such cross-reactions between pneumococci of the types 6A, 6B, and 35B on the one hand, and rabbit antisera to *H. influenzae* types a and b, on the other. Very faint reactions were observed between type b bacilli and rabbit antiserum to type 6B pneumococci (I).

The fact that most of the cross-reactions observed took place between the common pneumococcal type 6 and the two most common *H. influenzae* types a and b should be borne in mind when assessing the results of serological studies of these bacterial types.

The Relations between Encapsuled and Non-Capsulated Influenza Bacilli

Non-capsulated strains may arise by dissociation from encapsuled ones (88, 40, 23). Such *converted rough* strains generally display the decidedly rough form, with bacillary and polymorphous cultures and comparatively large and rough colonies. As to their antigenic composition, they do not essentially differ from the primarily non-capsulated, so-called *respiratory* strains. However, according to ENGBAEK (37), complete identity between the somatic antigens is more common among encapsuled

strains than among respiratory ones, which would seem to discourage the opinion that converted rough strains are wholly comparable to the respiratory strains.

The reversion from the non-capsulated to the encapsuled form had been reported only occasionally (88) when, recently, ALEXANDER & LEIDY (5, 6, 7) achieved the *in-vitro* transformation of converted rough cells to type-specific ones, and of type-specific cells to such of a new type. The changes were induced by desoxyribonucleic acids derived from type-specific cells. The exposure for three minutes of susceptible cells to the action of transforming agents was found sufficient for the transformation to occur, the cells being endowed with special receptors reacting immediately with the desoxyribonucleic acids. The number of susceptible cells varied considerably among separate strains and also among separate cultures of the same strain. Agglutinin against the receptor strain facilitated the development of the cells of the new type but was not necessary for the transformation to take place.

The possibility of a transformation *in vivo* from R to M forms was discussed by DOCHEZ, MILLS & KNEELAND (34), who observed a regular change in this direction in chimpanzees suffering from common-cold infection. A similar change was noted by BJUGGREN & TUNEVALL (14) in children suffering from upper respiratory infection with suppurative otitis. The explanation might be a shift during the infectious process of the balance between R and M forms in a mixed population, or a superinfection with an encapsuled strain or, finally, an *in-vivo* transformation of susceptible cells within a homogeneous R population. Two questions arise in connexion with the last-mentioned possibility, first, if there exist in the respiratory tract of healthy or ill subjects *H. influenzae* strains which contain cells susceptible to the action of transforming agents and, second, if transforming principles may come into contact with such cells under *in-vivo* conditions. In the following some observations as to the first question, which were reported in detail in a preceding paper (II), will be briefly reviewed.

Transformation experiments were arranged chiefly as described by ALEXANDER & LEIDY (6). Young Levinthal broth cultures of the strains to be tested were subjected to the action of transforming extracts from type-specific cells. In some experiments agglutinating antiserum to the tested strain was also added to the cultures. After incubation for 18—42 hours, a loop-ful of the culture was spread over a Levinthal agar plate which, after incubation for 10 hours, was examined for iridescent or in any other respect altered colonies. When variant R colonies were observed, they were subcultivated and subjected to new tests.

In a first series of experiments a number of converted rough (CR) strains proved transformable to type b and type d in the presence of agglutinating antiserum for the parent strain and transforming extract from type b or type d cells. Further, in one CR culture an auto-agglutinating variant emerged which in subsequent experiments could be made to develop type-specific cells also in the absence of antiserum. This observation confirmed the opinion of ALEXANDER & LEIDY (6), viz., that agglutinin against the parent strain is not necessary for the transformation but only for the consecutive development of the encapsuled cells.

The experimental conditions thus having proved appropriate, a number of respiratory (Re) strains were tested for transformability. Of these strains, 8 presented the type generally observed in CR strains, with bacillary, polymorphous cultures and comparatively large and rough colonies (Re—R), while the remaining 23 displayed the type generally found in Re strains, with short bacilli in fairly homogeneous cultures and small, comparatively smooth colonies (Re-S).

Of the Re-R strains three proved transformable just as CR strains. One of them had been isolated from a child who had just recovered from a type d *H. influenzae* otitis, one was obtained from aural secretion, and one from a child with maxillary sinusitis. The strains had been subcultured for at least two months without any signs of spontaneous conversion to the encapsuled state. Another Re-R strain which was obtained from aural discharge did not give rise to encapsuled cells but, in the presence of agglutinating antiserum, to non-capsulated cells of somewhat altered morphologic type and appreciably modified agglutination reactions. In subsequent tests this dissociant proved transformable. In the same manner behaved one of the Re-S strains, which had been isolated from the nasopharynx of a healthy child.

The experimental data thus supplied evidence that some *H. influenzae* strains isolated in the non-capsulated state contain, or may be made to develop, cells susceptible to transforming agents. Most of these strains represented the rough type which is common in converted rough strains, and one of them was furthermore isolated from a patient who had recently harboured type-specific cells. The question if these strains should be considered equivalent to converted rough ones and only therefore transformable or if, under appropriate conditions, all non-capsulated strains may develop susceptible cells must be left in abeyance.

It is a matter of interest, if any antigenic changes other than the acquisition of a capsular antigen were induced in rough *H. influenzae* cells by the transformation reaction. In an attempt to elucidate this problem, experiments with the gel precipitation method were performed, which will be exemplified here (V).

Antigen-antibody reactions in semisolid media, first observed by PETRIE (86), have been developed into an analytical method of wide applicability through the contributions of OUCHTERLONY (83, 84) and BJÖRKLUND (15, 16). The method involves the diffusion of antigens and antibodies in an agar gel towards one another from several diffusion centres consisting of basins spared-out in an agar plate. Where the reactants meet in optimal proportions for a flocculation to occur, precipitates emerge and form distinct lines in the medium. By special arrangements it is possible to analyze even complicated or multiple systems.

In the upper left basin of the plate shown in Figure 2 was placed a sodium carbonate extract (cp. p. 12) from a CR strain derived from a type d culture (CRd), and in the upper right basin a similar extract from a type b strain arisen from the former in a transformation. To each extract was added a supernate of a saline suspension of the strain in question, in order to ensure the presence of superficial and soluble antigens not represented in the extracts. The third basin was filled with rabbit antiserum to the type-specific strain. A system of precipitates emerged, where most lines were common to both extracts and formed arcades between the upper basins. Only one precipitate close to the basin of the type b extract found no counterpart on the other side. This precipitate could, according to other experiments, be considered to represent the capsular antigen, while the other lines, common to both extracts, corresponded to somatic antigens, which could not be shown to have changed in the transformation reaction.

The *in-vitro* Susceptibility to Chemotherapeutics

Most previous studies of the susceptibility of influenza bacilli have dealt mostly with the encapsuled form. The pathogenic significance of non-capsulated bacilli in the clinical material to be reported, together with the need of a study comprising all commonly employed chemotherapeutics and performed with a uniform technique, was the reason for the following study (III).

The tests were performed on Levinthal agar with the active compounds incorporated in serial twofold dilutions. The results were recorded after 10 hours at 37 C. The short incubation time was found to prevent significant deterioration or inactivation of the active compounds, which could be expected in this medium (93). However, the results must be assessed with consideration to the possible influence of peptones on the action of sulphonamide.

The results for the 63 strains tested are presented in Table 1, where encapsuled and non-capsulated strains are reported in common, except with regard to sulfametin, to which these two types reacted somewhat differently. It will be seen from the table, that inhibitory concentrations of sulfametin and penicillin for all strains tested may be obtained only by high dosage treatment. Most favourable was the relation between inhibitory concentrations and therapeutically obtainable ones for chloramphenicol.

A Complement Fixation Test for Haemophilus Influenzae Antibody

Previous studies of *H. influenzae* antibodies in human beings, generally performed by means of agglutination or complement fixation tests, have been liable to difficulties caused by the antigenic heterogenicity of this species. Adequate results could only be expected in acute infections, where the *H. influenzae* strain of the patient could be used as an antigen. Also this method might fail, the reason being changes of agglutinability often occurring in the strains on subcultivation (90). The antigenic

Table 1. Minimum inhibitory concentrations of six chemotherapeutics for 63 *H. influenzae* strains (Sulfametin mg/100 ml, penicillin IU/ml, others μg/ml).

Concentration	Sulfametin			Penicillin	Streptomycin	Aureomycin	Cloramphenicol	Terramycin
	C	N	T					
>8	1	12	13					
8	3	12	15					
4	3	1	4	1	16			
2	3	1	4	35	30			
1	4	3	7	22	13			
0.5	7	13	20	5	3	2	21	2
0.25					1	15	31	20
0.125						34	9	5
0.063						12	2	7

C = encapsuled strains. N = non-capsulated strains. T = totals.

The double lines through the columns indicate average concentration obtained in blood during treatment with standard dosage (42, 104, 43).

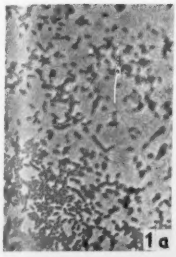
(Sulfametin = 2-sulfanilamido-5-methyl-1,3,4-thiodiazol ammonium.)

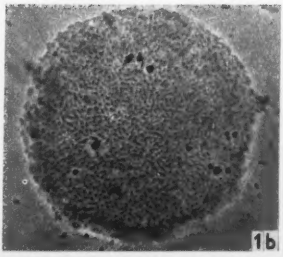
differences could also partly be eliminated by using antigens prepared from several strains (45, 29). Antibodies to the encapsuled form have been studied with capsular swelling reactions (4), cutaneous tests with capsular polysaccharide (32) and determinations of the bactericidal power of blood (41).

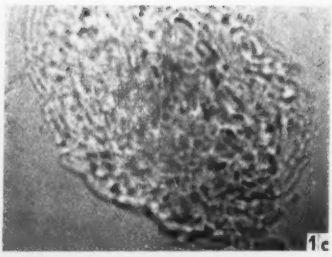
In order to develop a serological test recording immunization to *H. influenzae* in man, irrespective of the encapsulation and type of the immunizing strains, it was undertaken to prepare an extract from influenza bacilli, which contained as large a proportion as possible of antigenic components common to, or at least widely distributed within, the species (IV).

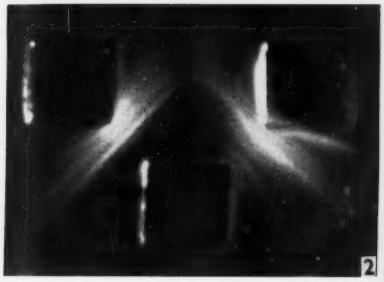
The existence of such fractions was disclosed by preliminary tests (V) where *H. influenzae* strains were cultivated, as described by OUCHTERLONY with regard to diphtheria bacilli (84), on trench plates with anti-type b rabbit antiserum diffusing from the trench. As will be seen from Figure 3, the emerging precipitates displayed identity reactions between all strains tested, which indicated the diffusion from the growing cultures of antigenic fractions common to the members of every pair of adjacent inocula.

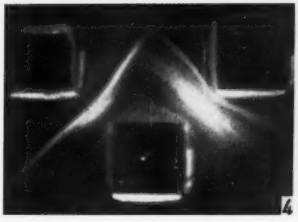
Subsequent studies (IV) resulted in the observation that organisms of the *genus Haemophilus*, unlike other bacteria tested, were completely dissolved by 1 % sodium carbonate solution in half an hour at room temperature. When the viscous solution formed was freed, by dialysis, from alkali and centrifuged at moderate speed, a clear, non-viscid supernate was obtained. In one pre-











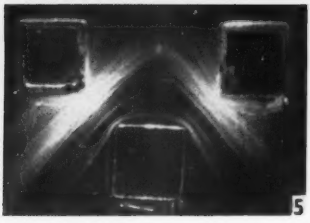


Fig. 1. Capsule swelling of slide cultures. a: Type d strain in normal rabbit serum. b: Same strain in type d rabbit serum (630 × phase contrast). c: Type b strain in type b antiserum (1200 × transmitted light).

Fig. 2. Gel precipitation in basin plate. Upper left: Extract from converted rough strain CRd. Upper right: Extract from transformed type b strain. Bottom: Antiserum to the type b strain.

Fig. 3. Trench plate. In the trench type b antiserum. The second and fifth linear inocula are type b strains. Note arcade formation.

Fig. 4. Gel precipitation in basin plate. Upper left: Growing type b strain. Upper right: Extract from same strain. Bottom: Type b antiserum.

Fig. 5. Upper left: Extract from converted rough strain CRb. Upper right: Extract from primarily rough strain. Bottom: Type b antiserum.

paration the dry weight was 8.3 mg/ml and the nitrogen content of the dry substance, 12.2 %.

Extracts thus prepared from encapsuled or non-capsulated influenza bacilli were precipitated by all tested anti-*H. influenzae* rabbit sera, though to a less degree by sera to encapsuled strains, which were prepared to contain chiefly anti-capsular immune bodies. Moderate or slight reactions were also obtained with some anti-pneumococcal sera but could be eliminated by diluting the extracts 1:4. No inhibition of the reactions due to antigen or antibody excess occurred within wide concentration ranges, probably while the reaction was complex, involving fractions for which the optimum proportions were obtained at different antigen and antibody dilutions.

On injection into rabbit, sodium carbonate extract proved a complete antigen, giving rise to agglutinin as well as precipitating and complement-fixing antibodies.

That most antigenic fractions which diffused from growing cultures were present also in the extracts is disclosed by Figure 4, where a type b strain was cultivated in the upper left basin, an extract from the same strain filled into the upper right, and anti-type b serum in the third basin (V). Most lines displayed identity reactions, but one sharp line at the basin of the growing strain, apparently caused by capsular substance, had no counterpart on the extract side, which indicated the absence in the extract of undeteriorated capsular polysaccharides. On the other hand, one line at the extract basin was absent at the growing culture, indicating the presence in the extract of additional components which did not diffuse from living cultures.

Further, comparisons by the gel precipitation method showed a close conformity between extracts from different strains. The fairly complete identity reaction presented in Figure 5 was displayed by extracts from a converted rough strain and a primarily rough one, when precipitated by type b antiserum.

As the precipitation reactions with rabbit immune sera were low-titered (1:32—1:128) and, besides, as the human sera were often turbid, which interfered with the readings, a complement fixation test was evolved in which sodium carbonate extract from a non-capsulated strain was used as an antigen. Details concerning the technique were given in a previous paper (IV). A rabbit immune serum was used as a standard. According to the results of double determinations, the reproducibility of the results was satisfactory (mean error —26 +35 %), and tests with immune sera to other bacteria showed no unspecific reactions.

In some children comprised in the clinical study, agglutination tests against the homologous strain as well as complement fixation tests (AHI) were performed on

repeated occasions during *H. influenzae* infections. The maximum agglutination titer was generally reached one or two weeks before the culmination of the AHI reaction, which observation will be discussed in the following. In apparently healthy *H. influenzae* carriers, the agglutination test was often negative, also when the AHI reaction was positive. This condition might partly be due to the greater specificity of the agglutination reaction, which prevented the detection of immunizations caused by strains other than the isolated one.

To summarize, the AHI reaction seemed to be a practicable method for the specific recording of antibodies to *H. influenzae*, irrespective of the encapsulation and type of the immunizing strains. As for most serological reactions, the results should be regarded as products of the intensity of the antibody response and the degree of conformity between the antigen employed and the strains once having effected the immunization. On account of the large proportion of species-comprising components in the antigen, however, the reaction should be an acceptable measure of *H. influenzae* immunization.

The Antipneumolysin Reaction

The methods commonly used for the demonstration of anti-pneumococcal antibodies in man have, when applied to small children, failed to disclose any evidence of immunization following pneumococcal infection (51, 24) or injection of pneumococcal vaccines (56, 76). The antipneumolysin reaction, as recording antibody to a soluble and easily diffusible antigen probably common to all pneumococcal types, could be expected to yield better informations in this connexion. This reaction was shown by Oker-Blom (81) to be applicable to clinical material. Hence, it was undertaken to work out a simple procedure for antipneumolysin tests in essential accordance with the technique used by Oker-Blom.

A pneumolysin suitable for the determinations could be prepared by the use of the same medium and the same procedure as those employed for streptolysin production. The details were given in a preceding report (VI). Also the titrations were performed largely as antistreptolysin determinations.

An anti-pneumococcal rabbit serum and, later on, a human serum were used for reference. The accepted antipneumolysin (APl) unit was likely to correspond to about 2/3 of the unit employed by Oker-Blom. The reproducibility of the results was fair, the mean error for a series of repeated determinations being —26 +35 %.

In immunization and neutralization experiments it was found that the specificity of the reaction was satisfactory. The non-specific neutralization between pneumolysin and antistreptolysin, demonstrated by Todd (101), was not found to play any significant rôle under the experimental conditions present in the tests. The same applied also to the cross-reaction between streptolysin and antipneumolysin.

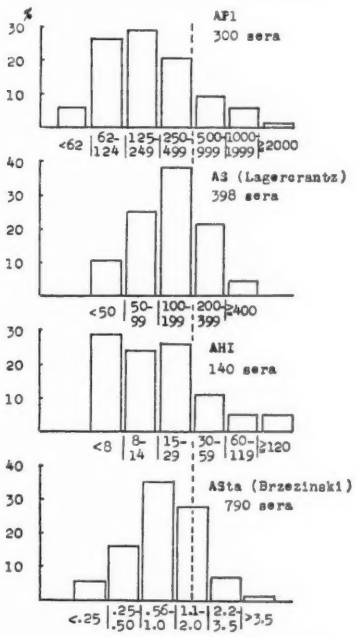


Fig. 6. Percentile antibody titer distribution in healthy adults. The dotted vertical line marks selected limits for "normal variation" in adults.

The Antibody Content in Sera of Apparently Healthy Adults

For the evaluation of APl and AHI rates of children, it was considered necessary to know the average antibody content in sera of adults. Hence, subjectively healthy persons not known to have suffered from infectious diseases during the preceding two or three months were examined for APl and AHI. The titer distributions are reported in Figure 6, where also the antistreptolysin (AS) and antistaphylolysin (ASta) titer distributions within comparable groups studied by LAGERCRANTZ (61) and BRZEZINSKI (19) are presented for comparison (IV, VI).

Where, for practical reasons, upper limits of "normal variation" of APl and AHI titers are applied in the following, they are selected according to this comparison, in order to be equivalent to the border values often used with regard to AS and ASta (111). The limits accepted were:

APl, 500 AU/ml; AS, 200 AU/ml; AHI, 30 units/ml; ASta, 1.4 AU/ml.

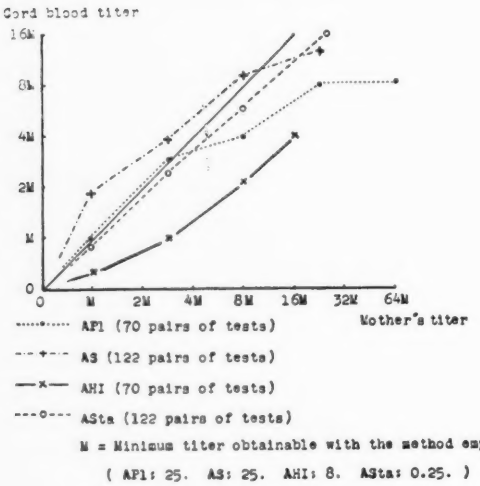


Fig. 7. Average ratio between mother's antibody titer and that observed in cord blood.

The Diaplacental Transfer of Antibodies

For the situation of the newborn child versus the infective agents to which it may be exposed, the inherited antibodies must be considered relevant. The diaplacental transfer of antibodies from mother to foetus has been found largely to take place from the fourth to the ninth month of gestation (108), whereas the passage is slow and irregular during the last month (107). Different antibodies seem to pass through the placenta with different degrees of ease. Thus, diphtheria (39, 109), tetanus (96), and scarlet fever (102) antitoxins, as well as antistreptolysin (100, 115, 48), antistaphylolysin (18), certain viral antibodies (54, 8, 105, 73, 94, 44), and antihyaluronidases (10) present about the same level in mother and child at confinement and are considered as easily passing the placenta. Certain antibacterial immune bodies, as Typhoid 'H' antibody (99) and *E. coli* agglutinin (1), have been found lacking, or almost lacking, in foetal blood, which suggests a poor permeability of the placenta for these antibodies. Finally, antibodies involved in the bactericidal process against encapsuled influenza bacilli seem to pass the placenta, though to a limited extent (41).

It has been investigated in connexion with the present study, how antipneumolysin and *H. influenzae* antibodies behave in this respect (IV, VI). The APl and AHI titers of healthy mothers and of blood from the umbilical cord were recorded, with the results shown in Figure 7. Also the results of corresponding AS and ASta determinations are presented for comparison. From the figure it will easily be seen that the three antilysin curves are largely parallel and indicate a close conformity between the antibody levels of mother and child. However, when the titer of the mother was very high, a

tendency towards lower rates in the cord blood was apparent, especially for API, as has already been reported by BERGQVIST (10). The explanation of this tendency is problematical. However, very high titers in the mother may generally be due to a recent acute infection. Thus, in many cases the antibody increase of the mother may have taken place during the last month of gestation, when the permeability of the placenta for antibodies is thought to be poor (107).

When compared with the antilysin curves, the AHI curve presents a different slope, suggesting that complement fixing antibodies to *H. influenzae* are not freely transferred on to the foetus, as has previously been found in respect of the bactericidal antibodies (41).

The inherited antibodies of the newborn are known to be eliminated during the first months of life, the 'half-lifetime' for diphtheria and Rh-antibodies being about 30 days (9, 112). During this period, the passively acquired antibodies are considered one of the causes of the feeble or delayed antibody formation which, however, is not as highly impaired as was thought formerly (108, 33, 50). The elimination is followed by a period of scanty or negative immune reactions, which lasts from about the third month to the commencement of active antibody formation. This production often begins very early for some antibodies but considerably later for others. These events have been closely followed with regard to e.g. antistaphylolysin (18, 65, 63), antistreptolysin (115), poliomyelitis antibodies (105), and bactericidal antibodies to *H. influenzae* (41).

The infection, in a strictly bacteriological sense, of infants with pathogenic bacteria is known often to take place already during the first weeks, which applies especially to children born in maternity hospitals (17). Pneumococci, haemolytic streptococci, and influenza bacilli have been found in the naso-pharynx in a large proportion of such infants two weeks after birth (52), and pyogenic staphylococci have been found almost consistently already after one week (28, 110).

The susceptibility to pathogenic bacteria of infants and small children and their reaction pattern on clinical infection have been found to vary with age and with the type of the pathogen (27). This variation is, *inter alia*, associated with differences as to the formation of antibodies to the separate pathogens. Pyogenic staphylococci are responsible for a number of infectious manifestations in very early infancy (22) but seem less significant as pathogens after the first year of life (75). This fact corresponds to the emerging of actively produced antistaphylolysin in many children already within the first half-year of life and to an adjustment during the following two or three years of the main titer level towards the range encountered in adults (18, 63).

Also pneumococci, often belonging to types of low pathogenicity to adults, are considered important pathogens in early infancy (36, 20, 27, 75), whereas the formation of anti-pneumococcal immune bodies has not been observed until the end of the second year of life (51, 24, 56, 76).

It is well known that encapsuled influenza bacilli, though scarcely pathogenic to adults, may give rise to severe infections in small children (87, 57, 3) and also that the bactericidal power of blood against such bacilli is lacking before the fourth year of life (41). Also the non-capsulated forms, however, have been found pathogenic to small children in connexion with respiratory infections and their purulent complications (57, 3, 37, 14). The immunological reactions to this form during childhood has not been subjected to study.

Unlike the aforementioned bacteria, haemolytic streptococci seem to increase their pathogenic significance for children with increasing age (36, 91, 75). In streptococcal diseases, the variation of the reaction pattern according to age is also more apparent

than in most other infections. The first confrontation of the very young child with haemolytic streptococci has been found generally to result in a so-called streptococcal fever, which is often comparatively mild but tends towards protraction and generalization. Subsequent infections approach the acute tonsillitis or scarlatinous type (91). According to several investigations the antibody reactions to haemolytic streptococci have been found weak in infancy and early childhood (115, 92) but later on increasing in strength, which has been explained as the result of several sub-optimal challenges which only eventually elicit full immune responses (92). The adjustment of the main titer level for antistreptolysin towards higher titers is slower than for antistaphylolysin (100, 115 48, 63). The adjustment results in titers higher on an average than those found in adults, but not until the later part of childhood (103).

The Antibody Content in Sera of Apparently Healthy Children

As no systematic investigations of antipneumolysin and antibodies to *H. influenzae* irrespective of capsulation have been carried out, a control group was examined with respect to APl and AHI and, for comparison, also for AS and ASta (IV, VI). This control group comprised 100 children below three years of age, apparently healthy or suffering from non-infectious diseases, and 120 healthy children aged from three to seven years. Most of the children within the latter age group were included in a study on the incidence of maxillary sinusitis and examined on three occasions. When the antibody rates of these children are reported in the present connexion as representing the average titer level of this age group, the last rate observed in every child is recorded, and values noted in children suffering from sinusitis due to the pathogen concerned are reported separately. The results of the determinations are demonstrated in Figure 8 (page 20).

Detectable amounts of all antibodies were generally found during the first two months, whereas negative reactions prevailed during the following two months. The rapid appearance of actively produced ASta and the comparatively slow reappearance of AS tallied well with previous observations, and the very few positive AHI reactions before the fourth year corresponded to the conditions known to apply to the bactericidal antibodies to encapsuled influenza bacilli. For APl, on the other hand, an early rise of the main titer level was recorded,[1] which resulted, in the last age group, in rates higher on an average than those found in adults. This observation deviated from previous results obtained with agglutination or capsule swelling reactions and indicated that the APl test revealed immunizations not disclosed by the reactions used previously.

The antibody rates within the control group will be further discussed in connexion with the sinusitis and otitis groups.

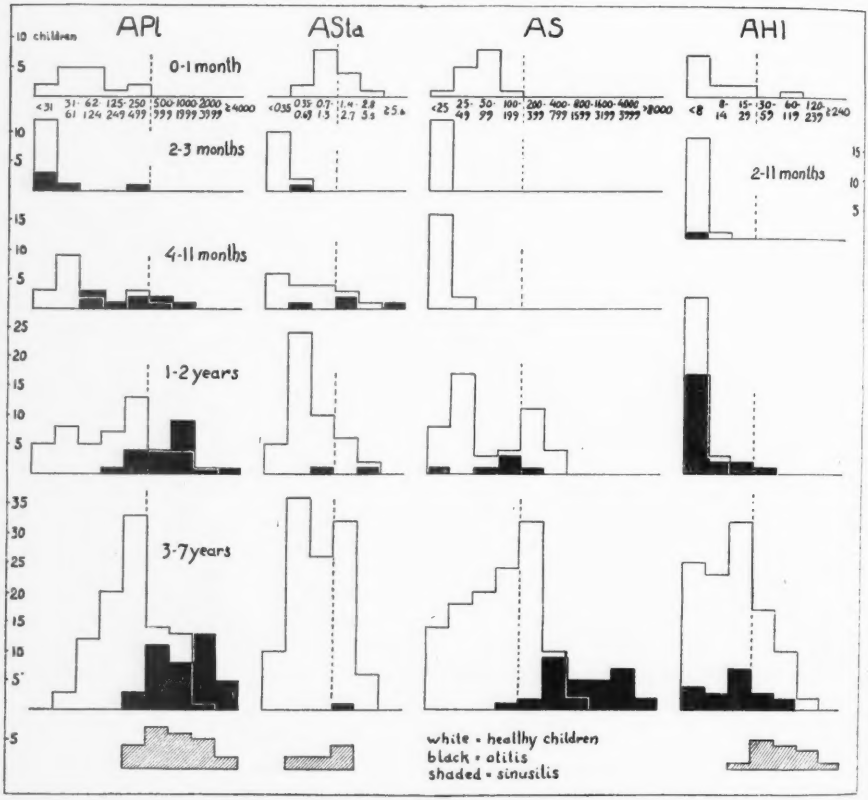


Fig. 8. Antibody titer distributions in healthy children, compared with maximum titers reached in otitis and maxillary sinusitis by the corresponding pathogen.

Sero-Bacteriological Investigations of Sinusitis in Children

It was early known from autopsies that inflammatory conditions of the paranasal cavities were common in children (82, 35). This observation was also confirmed by radiological examinations (59, 66). The selection of these materials, however, did not permit any conclusions as to the frequency of such conditions in the average population. A group studied radiologically by MARESH & WASHBURN (74) was more representative and displayed pathological antrum conditions in 34 % of all examinations. In follow-up studies of children from 3 to 7 years of age, selected to be representative of their age group, BJUGGREN, KRAEPELIEN & LIND (12, 13) obtained positive radiological findings in 50 % of the children on at least one occasion during the winter season.

Paranasal sinusitis in children has been investigated bacteriologically by several authors (31, 25) who all agree in considering pneumococci as the most frequent

pathogens. Staphylococci, though occasionally recovered from the antrum, have by some authors (25) been considered not to cause sinusitis. Also influenza bacilli have been found in antral discharges from children (26, 3, 37, 106).

Material and Methods

108 children between 3 and 7 years of age, selected to be representative for their age group (cp. BJUGGREN, KRAEPELIEN & LIND (13)) were comprised in the study (VII). All children were apparently healthy both when selected and subsequently at all examinations. They were examined on three occasions, two or three months apart, oto-rhinologically, with nose cultures and with radiological examination of the maxillary sinus. If pronounced sinus changes were found, sinus puncture was performed and the discharge examined bacteriologically. Further, a blood specimen was collected and tested for APl, AS, AHI, and ASta.

Details concerning the collecting of specimens, the culture methods, and the typing procedures were given in the preceding report (VII). Here will only be mentioned that antral discharges were collected by introducing a thin fiber swab through a trocar tube after puncture, in order to avoid contamination from the nasal meatus. A special cultivation technique was used which ensured the detection of influenza bacilli.

Of the haemolytic streptococci only those producing soluble haemolysin are termed 'streptococci' in the following, and of staphylococci, only those forming coagulase are named 'staphylococci'.

The three antibody rates observed in every child were taken as representing a positive serological reaction, if at least one value exceeded the accepted limit, unless each of the two later values were reduced to 50 % of the foregoing one. On the other hand, if any value amounted to four times the foregoing one, this was taken as a positive reaction, even if no value exceeded the limit. This evaluation aimed at the exclusion of antibody reactions due to infections occurring before the observation period, and likewise at the recording of antibody titer changes which might be significant despite having taken place at a low level.

The significance of differences in the titer distribution between separate groups were tested by the "chi-square" method.

Results

The nasal carrier rates for the pathogenic bacteria taken up for study, as well as the incidence rates of positive serological reactions, as evaluated from the three titers obtained in every child, are reported in Table 2. The AS reactions were more common than would be expected from the comparatively low incidence of streptococci. This might be explained, however, by the omitting of throat swab cultures, which generally yield streptococci more often than nasal cultures (98). On the other hand, the frequent occurrence of

Table 2. The nasal carrier rates for pathogenic bacteria within 108 apparently healthy children, and the incidences of positive serological reactions.

	Pneumo-cocci	Strepto-cocci	Influenza bacilli	Staphylo-cocci
Nasal carrier rates, %	53	27	19	69
Incidences of positive serological reactions to the pathogen concerned, %	56	44	28	37

staphylococci was not accompanied by a corresponding high incidence of positive ASta reactions. Thus, if a positive serological reaction as defined above is taken as a criterion necessary for an infectious process to have taken place, the pathogenic significance of the staphylococci did not correspond to their frequent occurrence, which made these organisms appear as "normal" inhabitants of the nasal mucosa.

The incidence of maxillary sinus processes was remarkably high. 31 % of all examinations resulted in pathological radiograms, and 60 % of the children presented positive findings on at least one occasion. 36 (33 %) of the children were punctured with positive result, 5 of which on more than one occasion, and 13 bilaterally. In all, 54 antral discharges were examined bacteriologically. The findings are reported in Table 3. Pneumococci and influenza bacilli prevailed, streptococci were never found, and staphylococci rather seldom in relation to their frequent occurrence on the nasal mucosa. The high incidence (27 %) of mixed infections was noteworthy and only partly due to different composition of the cultures from the two sides in bilateral processes. From the diagnostic point of view it was interesting that where pneumococci were present in the nose of a child with radiological sinus changes they were also recovered from the sinus 22 times out of 27, while the figures for streptococci were 0 out of 6, for influenza bacilli 4 out of 7, and for staphylococci only 3 out of 16. The degree of conformity between sinus and nose cultures may also be otherwise expressed. Pneumococcal sinus cultures were reflected by the nasal mucosa 22 times out of 26, *H. influenzae* cultures only 4 times out of 14, and staphylococcal cultures 3 times out of 8. Thus, especially influenza bacilli but also staphylococci were often harboured by inflamed sinuses without being recovered from the nose.

In Table 3 the incidences of positive serological reactions are reported separately for non-carriers, nasal carriers, and sinus carriers of the separate pathogenic bacteria. Positive reactions were more common among nasal carriers than in non-carriers of pneumococci, streptococci and influenza bacilli. This difference was especially pronounced when the bacteria were re-

Table 3. The incidences of positive serological reactions in non-carriers, nasal carriers, and sinus carriers of pathogenic bacteria.

	Pneumo-cocci	Pos. API %	Strepto-cocci	Pos. AS %	Influ-enza bacilli	Pos. AHI %	Staphy-lococci	Pos. ASta %
Non-carriers of the patho-gen concerned, No.....	51	37	79	38	80	19	32	33
Nasal carriers, No.	33	61*	29	62*	14	50**	68	41
Sinus carriers, No.	24	87**	0	—	14	79**	8	50

* = deviation from non-carriers statistically probable ($0.05 > P > 0.01$).
** = deviation from non-carriers significant ($P < 0.01$).

covered also from the sinus. For staphylococci the corresponding differences were not significant.

It should be added that the presence of catarrhal symptoms, without involvement of the paranasal cavities, was not associated with any increased frequency of elevated antibody rates.

To summarize, within a group of children suffering from no diseases other than occasional upper respiratory infections, 60 % were found to present maxillary sinusitis on at least one occasion. The prevalent pathogens found in antral discharges were pneumococci and influenza bacilli. Catarrhal conditions without paranasal sinus involvement were not associated with antibody titer increases. Nasal carriers of pathogenic bacteria often displayed high antibody titers, and this tendency was especially pronounced when the bacteria were harboured in inflamed sinus cavities. Thus, the antigenic influence of pathogenic bacteria was to a great extent exerted from inflammatory processes in the paranasal cavities.

Investigations of Suppurative Otitis in Children

The bacteria generally considered responsible for most cases of suppurative otitis are haemolytic streptococci, pneumococci, and pyogenic staphylococci (114). Pneumococci have been found to predominate during the first years of life, while haemolytic streptococci seem to be the prevailing pathogens of the later part of childhood (36, 27, 75). The significance of influenza bacilli as an otitis pathogen has been less appreciated though noticed by some authors, especially in small children (80, 37, 14). The serological reactions to otitic infections have been studied with respect to pneumococci and found to be weak during the first two years of life (38). CARLENS (21) has correlated the intensity of antibody formation in otitis due to Pneumococcus type 3 to the clinical picture of the disease. In a few children below one year of age, the lacking or feeble antibody production was associated with an insidious onset and a protracted course tending towards complications, i.e. resembling the clinical picture

common in adults over 30 years of age, who were also found to be poor antibody producers against this pneumococcal type. In older children and young adults the antibody formation was lively, the onset stormy, and the course uneventful.

A characteristic feature of otitis in early childhood is the tendency towards recurrence (68) which has not been eliminated by the introduction of sulphonamide and penicillin treatment (69, 49). It has been attributed to anatomical conditions, coinciding factors such as rhinopharyngitis, adenoids, allergy, and cross infections, and to antibiotic treatment over too short a period. The first study of the influence of the type of causal organism upon the relapsing tendency was reported by BJUGGREN & TUNEVALL (14) who found a relapse rate of 68% in children from which influenza bacilli were isolated on one or more occasions during the course of disease, as compared with 11.5% in other children.

Material and Methods

131 children of fairly even age distribution between two months and seven years of age, suffering from suppurative otitis, were included in the study and followed up with repeated aural and nasal cultures as well as serological examinations (VIII). To avoid over-representation of relapsing cases and the influence on the bacterial flora of antibiotic treatment prior to admission, no children who had sustained otitis previously during the last two months, or received treatment with chemotherapeutics during the same period, were comprised in the investigation. In order to ensure the detection of the primary pathogen — by aspiration of secretion from the middle ear prior to paracenteses, as was described in the previous report — only children in whom the tympanic membrane of at least one affected ear was not perforated, were included in the study.

Details concerning the bacteriological and serological technique were given in the previous paper (VIII).

The children were treated with sulphonamide or penicillin according to schedules mentioned in a preceding report (14). Out of the 131 children, 31 patients presented 42 relapses.

Results

The organisms found solely or prevailingly (>90 %) as primary pathogens, isolated in the first culture of relapses, and recovered as secondary invaders in subsequent cultures in all suppurations are reported, together with the nasal findings, in Table 4. The dominating part played by pneumococci in primary otitis was noteworthy, as well as the frequent occurrence of influenza bacilli. Staphylococci, on the other hand, were seldom found as primary pathogens. In relapses, influenza bacilli were more common than any other organism, viz. occuring in 38 %. As secondary invaders, finally, staphylococci were prevalent. It is also disclosed by the table that pathogenic bacteria other than those found in aural discharge were often recovered from the nose.

Table 4. Bacteria found solely or prevailingly (> 90 %) in the first aural culture in primary otitis (1) and in relapses (2), isolated as secondary invaders in subsequent cultures (3), and recovered in nasal cultures (4). Bacteria found in the nose without being recovered from aural discharge are indicated in brackets.

Age group, years	Bacteria	The bacteria concerned were found in			
		1. Cases No.	2. Relapses No.	3. Suppurations No.	4. Children No.
0—1	P	16*	7	1	20 (4)
	S	2	1	0	6 (4)
	H	4	9	1	9 (2)
	A	4*	0	10	10 (4)
	O	2	0	3	—
2—3	P	19	1	0	30 (15)
	S	8	4	3	10 (2)
	H	12	5	2	20 (8)
	A	0	2	9	20 (12)
	O	3	1	2	—
4—5	P	19*	2	1	22 (7)
	S	9*	1	1	7 (3)
	H	6	2	3	15 (7)
	A	1	0	10	16 (5)
	O	5	1	2	—
6—7	P	12	3	0	10 (2)
	S	11	1	1	7 (2)
	H	0	0	1	5 (0)
	A	0	0	5	7 (3)
	O	0	2	2	—
0—7	P	67 = 50 %	13 = 31 %	2 = 3 %	82 = 63 %
	S	30 = 21 %	7 = 17 %	5 = 9 %	30 = 23 %
	H	22 = 17 %	16 = 38 %	7 = 12 %	49 = 37 %
	A	5 = 4 %	2 = 5 %	34 = 60 %	53 = 40 %
	O	10 = 8 %	4 = 9 %	9 = 16 %	—

P = pneumococci S = streptococci H = influenza bacilli
A = staphylococci O = others, or no growth.
* = one case of bilateral otitis with another pathogen found in the opposite ear.

The distribution of the separate pathogens among children of different ages showed interesting differences. Pneumococci were common within all age groups, streptococci were rare in the small children and equalled the pneumococci only in the last age group, whereas influenza bacilli occurred most frequently in children from two to four years of age. The few findings of staphylococci were largely restricted to infants and very small children. In re-

currences, pneumococci often occurred during the first year of life, whereas influenza bacilli were frequent up to the age of three years. The age distribution of the primary pathogens will be seen also from Figure 8 (page 20).

Of the 45 *H. influenzae* strains isolated, 8 were encapsuled, and 4 of them belonged to type b.

The serological reactions observed in the children were reported in detail in the preceding paper (VIII). For the following discussion the general survey of the maximum titers reached in connexion with otitis will be more appropriate, as the values are subdivided according to age in the same way as the findings within the control group.

Some tendencies emerging from the graph should be stressed. Pneumococcal and staphylococcal otitis was in some cases associated with high antibody rates already from four months of age, and titers above the conventional limits, as defined previously, were almost obligatory from the second year of life. Streptococcal otitis, not observed in this material during the first year of life, produced titers over the same limit only after the third year. In *H. influenzae* otitis, detectable antibody appeared only occasionally before the same age. Further, it should be pointed out that the titer distribution of the *H. influenzae* otitis group did not deviate significantly from that of the control group, as did the titer distribution of the sinusitis group discussed previously.

Titer elevations for antibodies other than those corresponding to the pathogens isolated from the aural secretion were fairly common and often associated with the presence in nasal cultures of additional pathogenic bacteria. That only a proportion of the "heterologous" titer elevations was explained by the nasal findings may be due to the taking of only one nasal swab before the start of treatment and to the omitting of pharyngeal cultures, circumstances which might allow especially streptococci to escape detection (85, 97, 98). These observations made the otitic suppurations appear merely as parts of more wide-spread infections, which often involved other pathogenic bacteria than those causing the otitic processes. It should be mentioned in this connexion that the otitic disease was generally preceded by a period of upper respiratory infection which, especially prior to *H. influenzae* otitis, had often extended over a considerable time. The local otitic symptoms, on the other hand, had generally lasted only one or two days before admission.

The clinical picture of the *H. influenzae* otitis, more closely described in the preceding report (VIII), was characterized by the often prolonged precursory catarrhalic symptoms and its mild but frequently prolonged course with a pronounced relapse tendency. The mucus was often viscid or stringy, as was the nasal discharge of the frequently concomitant rhinitis.

The results of chemotherapy tallied well with the *in-vitro* susceptibility

of the organisms isolated from the discharge. However, the presence in the discharge of staphylococci, even of high resistance to the drug employed, was not found to interfere with the therapeutic results, which suggested that staphylococci were not alone able to maintain a suppuration. No influence of the treatment on antibody formation could be demonstrated, probably because chemotherapy was generally started comparatively late in the course of disease, that is to say if the precursory disease was taken into calculation.

The incidence of recurrences was connected with some circumstances which will be briefly surveyed. The significance of age was evident already from Table 4, as well as the prominent rôle of influenza bacilli. Within the group of relapses due to pathogens isolated from the patient already during the preceding suppuration, the proportion of *H. influenzae* relapses was still larger, or 54 %. This fact may partly be due to the barely moderate sensitivity of influenza bacilli to the chemotherapeutics employed, which resulted only in the cessation of the suppuration but not in the elimination of the bacilli from the respiratory tract and the paranasal cavities, where they are known to persist for considerable periods (3) in spite of sulphonamide or penicillin treatment. The relapses will be further discussed in the following section.

General Discussion

The studies summarized in the present paper aimed not only at a bacteriological analysis of paranasal sinusitis and otitis in children and the serological verifying of the aetiological information obtained. It was also endeavoured to select the clinical material and to deal with it in such a manner as to make it applicable as a basis for a comprehensive study of the special conditions associated with bacterial infections in childhood. In the following the investigation will be discussed largely from this point of view.

The material

The elaborate clinical and sero-bacteriological follow-up of every child comprised in the investigation prohibited the extension of the study to large groups. The limited size of the clinical material called for great care at the evaluation of the collected data. The age distribution of the otitis material and of the control group was fairly even, whereas the study of sinus infections, on account of the inconsiderable pneumatization of the sinuses before this age, was restricted to children over the age of three years. Most of the children below three years within the control group and the children suffering from otitis were selected at random from the heterogeneous clienteles of large clinics, whereas the remainder of the control group and the children with sinusitis were selected in equal proportions among "home-children", nursery-children, who, according to HESSELVIK (55), are especially liable to respiratory infections, and school-children. All groups were collected from October to April in order to diminish the differences due to seasonal factors. The special precautions in order to avoid biasing of the otitis material were already mentioned. — During the observation period 3 855 cases of scarlet fever were observed in Stockholm, with the maximum in October (1 105 cases) and the minimum in April (240 cases).

The methods

Great care was taken, and special methods were used, in order to ensure the representativity of the specimens form the naso-pharynx, the sinuses, and middle ears. However, the taking of only one nasal swab in the otitis cases prior to treatment, as well as the omission of throat swabs, might have allowed especially haemolytic streptococci to escape detection, which was also suggested by the serological results.

While the bacteriological technique, which involved the selective cultivation of influenza bacilli by use of penicillin, needs no comments, the serological methods will be briefly discussed. The API reaction was subjected to tests for specificity and not found liable to any significant overlapping. Its specificity was further confirmed by the clinical study, where the API rates generally varied independent of the other antibody reactions. For a comparative study of the present type, where the AS and ASta reactions were employed simultaneously, the API reaction was suitable since it is essentially analogous to these tests.

Unlike the antilysin reactions, the AHI test records immunizations to somatic antigens, many of which are released from the bacterial cell only on its disintegration. However, as no soluble and easily diffusible antigens comparable to lysins, toxins, or hyaluronidases could be demonstrated in influenza bacilli, the essential difference mentioned does not necessarily make the reaction less adequate for recording anti-*H. influenzae* immunity. The feeble or lacking immune responses which were demonstrated by the AHI reaction may on the contrary be the result of the weak antigenic stimulation exerted by an organism lacking easily diffusing antigens and bound to the surface of the mucous membranes. It was observed, in immunized rabbits, that the reaction to vaccination, which results in a more effective contact between the antigens and the host tissues, was high-titered. As a parallel to this observation it may be mentioned that pertussis vaccination has been found to elicit antibody responses more vigorous than those observed in pertussis convalescents (77). However, the association of the AHI reactions partly to antigens likely to be deeply situated in the bacterial body may be one reason why the occurrence of AHI antibodies was delayed when compared with the emerging of agglutinins.

The aetiology of paranasal sinusitis and suppurative otitis

Maxillary sinusitis and suppurative otitis, being inflammatory processes in normally sterile cavities, where the aetiological significance of recovered pathogenic bacteria would seem to be indubitable, and occurring frequently in children, were suitable subjects of the study as representing the infections of the upper respiratory tract with its adjunct structures. The results of the bacteriological examination will be surveyed here only when deviating from previous experience or relating to debated questions. Thus, the part played by influenza bacilli in otitis and in sinusitis should be stressed and, on the other hand, the very slight significance of staphylococci as primary pathogens in otitis. As suggesting a different liability of separate sites to infections by the same pathogen, it may be mentioned that, during a scarlet fever epidemic

when streptococcal nose carriers were common within the observed group, and streptococcal otitis was often found in children of the same age, no single case of streptococcal sinusitis was encountered.

The incidence of encapsuled influenza bacilli was higher in suppurative otitis (8 out of 34 patients) than in apparently healthy children, even if presenting maxillary sinusitis (2 out of 38 children). This difference ($P = 0.05$) suggested that the occurrence of otitic suppurations, but not of maxillary empyema, was associated with the presence of encapsuled bacilli. This might be explained by a greater ability of encapsuled bacilli to cause otitic suppurations, or by a furtherance of the development of encapsuled bacilli by the same factors which promote an otitic suppuration. The possibility of transferring type specificity to non-capsulated strains might be relevant for this observation. A statement of ALEXANDER, ELLIS, & LEIDY (4) is interesting in this connexion, viz. that "the majority of influenzal meningitis patients show clinical signs of paranasal sinusitis, but evidence for direct extension from this source is lacking. — Purulent otitis media is an unusual forerunner of influenzal meningitis but a very frequent component of the infection in those patients who fail to respond to therapeutic measures or in those who have been sick for a long time before being treated."

Further, the age-conditioned variation of the incidences of separate pathogens was noteworthy. Staphylococcal otitis was largely restricted to the first year of life, *H. influenzae* otitis occurred up to four years of age, and pneumococcal otitis was frequent in all age groups, while, on the other hand, streptococcal otitis grew more common with increasing age.

The antibody formation in different ages

The view that the enhancement of the antibody responses with increasing age is the result of a cumulative effect of several suboptimal antigenic challenges, which was especially clearly expressed by RANTZ, MARONEY & DICAPRIO (92) with respect to streptococci, tallies well with the observations made in the present study, also with regard to the other pathogens investigated. Thus, the very early emerging of actively produced ASta in the infants of the control group ought to be correlated to the occurrence of staphylococci as almost "normal" inhabitants of the nasal mucosa already from the first weeks after birth and the not infrequent staphylococcal infections simultaneously observed in the Sachs' Hospital for Children (62, 64) from which this part of the control group was mainly collected. The very early infection in a bacteriological sense, probably not seldom associated with an additional clinical infection, may account for the rapid increase of the main ASta titer level.

The evolution of the reactivity towards pneumococci, as measured by the API reaction, was largely parallel to that of the ASta reactions, while the corresponding progress for the AS reactions was considerably delayed. This difference agreed with the lower carrier rates for streptococci and the comparative rareness of streptococcal infections in the lower age groups of the clinical material. The difference was reflected in the slower change of the mean titer level towards higher values as well as in the weakness of the AS reactions in acute infectious processes until the fourth year.

When applying the same considerations to the AHI reaction, the different nature of this test must be taken into account. The feeble or lacking immune responses revealed by the AHI test as well as the rareness of detectable immunizations up to the age of three years, despite the frequent occurrence of *H. influenzae* infections in infants and small children, have already been discussed. Another observation should also be mentioned in this connexion, viz.. that the antibody rates to *H. influenzae* were higher in upper respiratory infections associated with sinusitis than in such accompanied by otitis media. This difference, though it was partly due to lower initial values in children with otitis than in the other children, would also seem to agree with the proposed explanation. The maxillary antrum is liable to occlusion when inflamed— which may even result in empyemas under pressure — and likely to afford good conditions for the absorption of antigenic substances from disintegrating bacteria. Also the high incidence in the antrum of mixed infections, which often involved hyaluronidase-producing bacteria, might be important with consideration to the possibility of an increased pressure, and especially if the infectious process under the given conditions is deeply propagated in the submucosal tissue (53, 11). This condition may be one of the reasons for the observation that the AHI rates of eight sinusitis cases with influenza bacilli in pure culture did not reach 120, whereas this value, or higher ones, was observed in four cases out of six, where pneumococci or staphylococci had been found in the sinus simultaneously or at the preceding examination ($P = 0.04$). The frequent occurrence of maxillary sinusitis, together with the often intense antibody responses observed in this condition, renders plausible that the paranasal sinuses play an important rôle in the development of immunity in children to the respiratory pathogens.

The correlation between the relapsing tendency and the antibody formation in otitis

The influence of the treatment on the incidence of otitis relapses has already been discussed with regard to influenza bacilli. However, also other factors may be involved in the processes resulting in a recurrence. The

different reaction patterns to infection, as discussed in respect of streptococcal diseases by POWERS & BOISVERT (91) and with regard to pneumococcal otitis by CARLENS (21), and correlated by him to the intensity of the immune responses, are suggestive in this connexion. In the present material the relapses were closely correlated to age, as was the power to produce antibody. Thus, pneumococcal relapses occurred chiefly during the first year of life, *H. influenzae* relapses up to the age of 4 years, and streptococcal recurrences occasionally in all age groups. These periods coincided with the periods of poor antibody formation against each of these pathogens. The significance of deficient antibody production must, however, be studied within a narrowly limited age group in order to eliminate the age factor from the calculation. For such a study only the pneumococcal relapses of the children aged from 3 to 11 months furnished evaluable data. Among 11 primary pneumococcal suppurations within this group, 4 cases suffered from recurrences, one case relapsing twice, whereas 7 cases escaped recurrence. Of the four relapsing cases and the recurrence followed by another suppuration, none presented an API rate above 125, whereas 6 cases out of 7 escaping relapses exceeded this limit. This difference was statistically probable ($P=0.02$). It may also be pointed out in this connexion, though relating to primary otitis, that in the children above three years of age the AHI reaction was 10 times out of 13 negative in the first test from *H. influenzae* otitis cases, while the corresponding figures for healthy children of the same age was 17 out of 83 ($P<0.01$). Thus, together with the very small children, also those older children who lacked detectable antibody were especially liable to *H. influenzae* otitis.

The antigenic effect displayed by respiratory infections was not found to be significantly increased by the supervention of a suppurative otitis, which furthermore often arose comparatively late in the course of disease. Nor could any influence on the antibody formation in otitis be attributed to conditions connected with antibiotic treatment. Consequently, suppurative otitis should, as tending to para- and post-otitic complications, be early and adequately controlled with chemotherapeutics. This, especially in small children, requires bateriological diagnosis.

Summary

1. The type characteristics of *H. influenzae* were studied. The typing procedure for encapsuled strains was improved e.g. by employing slide cultures for capsule swelling tests. The serological cross-reaction with encapsuled pneumococci, especially type 6, was confirmed. No hyaluronidase production could be demonstrated in *H. influenzae* (I).

2. The relations between encapsuled, converted rough, and primarily rough strains of *H. influenzae* were studied in transformation experiments. By varying the experimental conditions and watching carefully for all emerging variants, including non-capsulated ones, which were subsequently tested in new experiments, it was possible to transfer capsule formation ability and type specificity to a number of strains isolated in the non-capsulated state (II). No change of the antigenic composition of the bacilli could be observed, by gel precipitation experiments, in transformed strains, except that the acquisition of a capsular antigen was demonstrated (V).

3. The susceptibility of *H. influenzae* to six commonly used chemotherapeutics was investigated. Aureomycin, chloramphenicol, and terramycin were found most effective (III).

4. By the gel precipitation method, the presence in influenza bacilli of antigenic factors common to, or widely distributed within, the species was demonstrated (V). Unlike other tested bacteria, haemophilic organisms were easily dissolved by 1% sodium carbonate solution, which was used for the preparation of an extract rich in the widely distributed antigenic factors (IV).

5. The use of a sodium carbonate extract as the antigen of a complement fixation test (AHI) resulted in a reaction which, unlike tests used formerly for the recording of *H. influenzae* immunity, was fairly independent of the type and capsulation of the immunizing strains (IV).

6. A convenient routine method for the determination of antipneumolysin (APl) was worked out. No significant overlapping versus antistreptolysin was noted (VI).

7. The transfer of APl, AS, AHI, and ASta from mother to foetus was studied. The transmission was more effective with regard to the antilysins than to the antibacterial complement fixing antibodies to *H. influenzae* (IV,VI).

8. The content of APl, AS, AHI, and ASta in infants and children of different ages was found to be rising with increasing age, though with different rates for the separate antibodies (IV, VI).

9. Maxillary sinusitis was found on one or more occasions during one winter season in 60% of apparently healthy children. Pneumococci and influenza bacilli were the prevailing pathogens, whereas staphylococci were comparatively rare. Haemolytic streptococci were never found. These observations deviated from most previous investigations and could be ascribed to the adequate methods used for collecting and examining the antral discharges. The antibody titers in nasal carriers of the separate pathogens were higher than in non-carriers but significantly increased only when the pathogens were present in inflamed sinuses. The importance of the immunization exerted by sinus processes is stressed (VII).

10. In suppurative otitis, pneumococci were common in all ages up to seven years, while pyogenic staphylococci as primary pathogens were rare and largely restricted to infants and very small children. Influenza bacilli were frequent up to four years of age, and haemolytic streptococci increased their incidence with increasing age. This prevalence of different pathogens in the separate ages was paralleled by a corresponding difference in the evolution of the ability to produce antibodies to the same

pathogens. The respiratory infection which generally preceded the otitis was found often to involve bacteria other than those found in the aural secretion. The *H. influenzae* otitis displayed a characteristic picture and tended towards recurrence. Further, the significance of age and of treatment for the occurrence of relapses was studied (VIII).

11. In the discussion, the pathogenicity of encapsuled influenza bacilli with regard to otitis is compared to that of non-capsulated strains. The main antibody titer levels and the antibody responses to acute infections in different ages are correlated to the variation according to age of the incidence of infections by the corresponding pathogens. The high antibody titers, and especially the high AHI rates, observed in maxillary sinusitis are discussed. The antibody production is pointed out as one of the factors which influence the course of infections and, with regard to otitis, the incidence of relapses.

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Head: PROF. BO VAHLQUIST

THE IRON-BINDING CAPACITY OF SERUM IN INFANTS AND CHILDREN

By

BENGT HAGBERG

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INTRODUCTION

The available, non-haemoglobin iron in the body is attached to special proteins with iron-combining properties. The knowledge of their presence, nature, and function dates mainly from the years 1945—1950.

Iron is stored in an easily available form as ferritin, which consists of the protein apoferritin with attached iron. Normally this complex does not appear in the blood, where a special iron-binding plasma protein is to be found instead. It is called transferrin (siderophilin, beta$_1$-metal-combining globulin). The small but important amount of non-haemoglobin iron in the blood, the plasma or serum iron, is combined with this protein for transport in the blood. Only about one third of the total iron-binding capacity of the transferrin is, however, saturated at ordinary serum iron levels. The rest exists in an iron-free, unsaturated form. The concentrations of serum iron and transferrin and their mutual relationship become altered in characteristic ways in physiological and pathological changes in the iron balance. They are probably closely associated with the distribution, storage, and mobilization, of iron. Combined determinations of the two factors will therefore give a far better understanding of the actual state of the iron metabolism than measurement of the serum iron alone.

After a survey of the physiology of iron from the fetus to the adult an account is given in this work of various changes in the serum iron and the iron-binding capacity of infants and children in health, iron deficiency, and infections. Special emphasis is laid on the physiological variations during early infancy, when the amount of storage iron is known to undergo more changes than at any other time in later life.

Abbreviations

TIBC = Total iron-binding capacity.
UIBC = Unsaturated iron-binding capacity.
SI = Serum iron.
ESR = Erythrocyte sedimentation rate (micro-method).
RES = Reticulo-endothelial system.

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CHAPTER I

General Review of Iron Metabolism

Although the total amount of iron in the body is extremely small, the fact that it is an essential constituent of haemoglobin, myoglobin, and certain enzymes, makes it an element of vital importance in metabolic processes. Its chief functions are concerned with the transport of oxygen to the tissues, and cellular respiration.

1. Distribution of iron in the body

The total iron in the body of an adult man amounts to 4—5 g. Not less than about 60 per cent consists of haemoglobin iron, about 5—10 per cent is found in the form of myoglobin, and of the rest 20—25 per cent is storage iron easily available for haemoglobin production. Less than one per cent is found in the extremely important enzymes cytochromes, cytochrome oxidase, peroxidase, and catalase. The remainder is practically all fixed cellular iron with but little known function. The small quantity of transport iron, the plasma iron, represents about 0.1 per cent of the total body iron (for references see HEMMELER, 1951; WINTROBE, 1952; WIDDOWSON *et al.*, 1951; FINCH *et al.*, 1950; HASKINS *et al.*, 1952).

2. Ferritin and haemosiderin, and the storage of iron

The term 'storage iron' means the iron which can be mobilized as required from different tissues for the formation of haemoglobin. Ordinarily the storage iron is not utilized, but when production of red cells exceeds destruction, iron is immediately mobilized from the depots. On the other hand excess iron will be transported to the depots when the destruction of red cells increases in comparison with the new production. The iron is stored intracellularly in iron-protein complexes, mainly as ferritin, but to some extent also as haemosiderin.

Ferritin (LAUFBERGER, 1937; GRANICK, 1942—1951) consists of a protein, apoferritin (mol. wt. 460000), and up to 23 per cent of iron. The colourless apoferritin is synthesized by the cells only when needed. It does not usually appear in free form. The iron is physically bound to apoferritin as ferric hydrox-

ide 'micelles' or 'clusters' (GRANICK, 1946). Ferritin can be produced in various cells and organs. Its formation seems to be the defence mechanism against an excess of free iron ions, and also the physiological means of storing iron in the body. Iron as ferritin is easily available and easily soluble, because apoferritin apparently constitutes a protective colloid for the low-molecular ferric hydroxide micelles. These are prevented from subsequently condensing and enlarging, and thus from precipitating in the form of an insoluble ferric hydroxide gel.

Haemosiderin has been regarded as an abnormal stage of iron deposition in the cells (GRANICK, 1949; SCHWIETZER, 1951). Another opinion has been expressed by FINCH and associates (1950), and others, who hold that the formation of haemosiderin is a second physiological form of iron storage. It is, however, mainly resorted to when there is excessive supply of iron to the depots, the formation of apoferritin being thought to be insufficient for transformation of large amounts of iron into ferritin. Haemosiderin, which contains less protein and more iron, is then formed. When its iron content increases the haemosiderin is thought to become more stable and insoluble. Though ferritin-iron is thus presumed to be more readily available than haemosiderin-iron, both become depleted from the tissues when iron is needed for erythropoiesis (FINCH *et al.*, 1950).

The liver contains most of the iron stored in the body. A smaller part of this iron fraction is found in the spleen and the bone marrow. Recently HASKINS *et al.*, 1952, have shown, by studying erythropoiesis following repeated phlebotomy in four men, that in all 1200—1500 mg of iron was available for the formation of haemoglobin. The depleted stores of iron were shown to be replaced very slowly. When no extra iron was given these were not even adequately filled one year later. In the replenishment of the iron depots it seems to be unimportant whether iron is administered by the oral or the parenteral route, as the ultimate distribution is found to be practically the same (FINCH *et al.*, 1950).

3. Excretion and absorption of iron

It is almost impossible to eliminate physiologically iron once incorporated in the body (MCCANCE & WIDDOWSON, 1937, 1938; BARER & FOWLER, 1937; HAHN *et al.*, 1939; and others). In the normal adult man an average loss of about 1 mg per day can be reckoned with, in the normal adult woman 2—3 mg depending on the menstrual loss and the frequency of pregnancies. The diurnal urinary excretion of iron is about ½ mg, although many times as much iron passes through the kidneys during the day. Minute quantities are lost in the bile (GREENBERG *et al.*, 1943; HAWKINS & HAHN, 1944), and almost in-

significant amounts in shed intestinal and dermal cells. Evidently there is no colonic excretion of iron (McCance & Widdowson, 1943).

The mechanism of iron absorption seems to be complicated, and is only partially known. Iron in the food occurs usually as colloidal ferric hydroxide, and is reduced in the stomach to ferrous iron. The iron is better absorbed in this divalent than in trivalent form, as has been demonstrated in studies with radio-active iron (Moore *et al.*, 1944; Hahn *et al.*, 1945). Investigations using auto-radiography have definitely proved that most of the absorption occurs in the duodenum near to the pylorus (Endicott *et al.*, 1949). Iron can also be absorbed from the stomach (Vahlquist *et al.*, 1945) and from more distal parts of the intestine (Granick, 1946). Above all, absorption is favoured by a diet with a low content of phosphates (Gillman & Gillman, 1947; Hegsted *et al.*, 1949), with an excess of ascorbic acid (Moore & Dubach, 1951; and others), by the addition of copper to the diet (Chase, *et al.*, 1952), and by a deficiency of protein (Schwietzer, 1951).

The amounts of iron absorbed daily under physiological conditions are small. Preliminary studies with radio-active iron incorporated in normal foods have shown that commonly less than 10 per cent of the iron in the food is absorbed (Moore & Dubach, 1951). Thus in order to maintain iron-balance 10—30 mg iron per day must be introduced into the intestinal tract with the food. The results of the same investigation also suggest that iron-deficient subjects can absorb no more iron from the food than healthy persons. On the other hand, inorganic iron preparations are better absorbed by subjects with an increased demand for iron (Hahn *et al.*, 1943, 1951; and others). A surplus of iron in sufficient amounts can also increase the absorption in healthy individuals to some extent, but the greater the dose the smaller the proportion absorbed. Some regulative mechanism must be present, particularly as the body is practically unable to eliminate iron, i.e. to regulate the iron balance by excretion.

The saturation of the iron depots is considered to be the essential factor in the control of iron absorption (Balfour *et al.*, 1942), but the mechanism is still obscure. One suggestion is that control may take place through a disturbed equilibrium of the iron ion activity in the iron stores — plasma — intestinal-mucosa system, possibly regulated by alterations in the transferrin level (Laurell 1947, 1951, 1952). Granick (1946), on the other hand, considers the formation of ferritin in the cells of the intestinal mucosa to be of central importance in the genesis of the 'mucosal bloc'. In later papers he seems, however, to be more reserved towards this assumption and says (1951): 'whether changes in ferritin concentration are directly connected with the mucosal bloc, or whether the presence of much ferritin merely indicates that the mucosal cells are saturated with respect to some iron compounds more closely allied with the mucosal bloc, has not been determined'.

4. The plasma transport of iron

In 1925 FONTÉS and THIVOLLES called attention to the fact that a small amount of non-haemoglobin-bound iron was present in plasma. The existence of the plasma iron, which amounts to about 0.1 mg per 100 ml was confirmed and further elucidated during the next two years (BARKAN, 1927; and others). Comprehensive experimental and clinical investigations on serum iron are found in particular in monographs by THOENES & ASCHAFFENBURG (1934), HEILMEYER & PLÖTNER (1937), SKOUGE (1939), VAHLQUIST (1941), BÜCHMANN (1941), VANOTTI & DELACHAUX (1942 and 1949), LAURELL (1947 and 1952), and HEMMELER (1951).

The plasma iron merits attention, mainly because of its central position in the transport of iron by the intermediate haemoglobin metabolism. The plasma iron functions mainly as a link in a closed cyclic process (haemoglobin—plasma-iron—haemoglobin) which is more or less independent of absorption, excretion, and storage. Every day 20—24 mg of iron is transported as plasma iron to the bone marrow solely for the production of haemoglobin. According to HUFF *et al.* (1950), the total average amount of iron normally turned over as plasma iron has been calculated to 27 mg per day. This seems to be regulated mainly by the rate of erythropoiesis.

The combination of iron in plasma has received much attention since the discovery of the serum iron. BARKAN (1927) was the first to show that the iron of serum is non-dialysable at the pH of the blood, but dialysable after acidification. He also observed that iron does not appear in an ultrafiltrate unless acidified (BARKAN, 1933). It was soon confirmed that the iron in serum is protein-bound. Fractionation of sera with half-saturated ammonium sulphate precipitates the iron quantitatively with the globulins (STARKENSTEIN & HARVALIK, 1933; BARKAN & SCHALES, 1937). Similar investigations using natural plasma containing radio-active iron indicated on the other hand that the iron here is bound to the albumin fraction (YOSHIKAWA *et al.*, 1942). Using electrophoresis VAHLQUIST (1941) found that iron in the serum wandered with both the albumins and the globulins in natural serum, but mainly with the alpha- and beta-globulins.

In 1945 HOLMBERG & LAURELL demonstrated that serum from healthy subjects is capable of combining with added iron up to about 300 gamma per cent in firmly-bound form, which, it was suggested, was an iron-protein complex. The figures of HOLMBERG & LAURELL agreed with the serum iron levels due to the 'braking effect' of WALDENSTRÖM (1944). He and SKOUGE (1939) thus found that the iron concentration in serum after injections of 10 mg Fe^{+++} as a simple salt solution never reached the expected values, but stopped at about 300 gamma per cent. This saturation limit of serum was further in-

vestigated by LAURELL (1947). He found among other things that iron added to serum above the iron-binding capacity is dialysable and can be adsorbed onto AlC_γ.

Ultimately it was obvious that the values at the braking point, the saturation limit, and the total iron-binding capacity of the special iron-binding protein, transferrin, are due to the same factor.

5. Transferrin, the iron-binding component in plasma

During their investigations on the saturation limit HOLMBERG & LAURELL (1945) observed that ferrous iron added to serum gives a change in serum colour from yellow or yellow-green to yellow-red. By photometry at about 5000 Å this change in serum colour was measured as a definite increase in extinction, which developed successively on repeated addition of small amounts of iron until the saturation limit was reached. Independently SCHADE & CAROLINE (1946) made the same observations. They also demonstrated fully that the change in colour was due to the formation of a special iron-protein complex. This was first shown to be concentrated in the fraction IV–3,4 by SCHADE & CAROLINE. Further subfractionation, however, led to the concentration of the iron-binding component into the new fraction IV–7 (SURGENOR *et al.*, 1949). Finally, the component was crystallized in a stable iron-saturated state by LAURELL & INGELMANN (1947), and in a mainly iron-free form by KOECHLIN (SURGENOR *et al.*, 1949).

The iron-binding protein appears in the literature under different names. The synonyms nowadays used are '$beta_1$-metal-combining globulin' (SURGENOR *et al.*, 1949), 'siderophilin' (SCHADE *et al.*, 1949), and 'transferrin' (HOLMBERG & LAURELL, 1947). Because it seems to be the best the last name will be used here.

Iron-free transferrin is colourless, whereas the iron-protein complex is salmon-coloured with a broad absorption maximum at 4700 Å. This fact has been very useful in spectrophotometric determinations of the iron-binding capacity in clinical practice (RATH & FINCH, 1949; CARTWRIGHT & WINTROBE, 1949).

Transferrin (mol. wt. about 90000) can bind 1.25 gamma of iron per milligram protein, corresponding to two atoms of iron per molecule. Normal human plasma has been estimated to contain about 2½ g transferrin per litre plasma, and is capable of binding approximately 3 mg of iron. Transferrin iron is always in the ferric state and ionic form, as shown by MICHAELIS (see SURGENOR *et al.*, 1949) in measurement of the magnetic susceptibility. Whether ferric or ferrous iron is added, however, the same amount is taken up in complex form.

A generally accepted opinion on the uptake of iron by plasma is that trans-

ferrin takes up free iron ions from destroyed haemoglobin, iron stores, and cells of the intestinal mucosa. More controversial views exist about the liberation of iron from plasma. One assumption is that the whole Fe-transferrin molecule leaves the blood stream (FLEXNER *et al.*, 1948). The facts available do not support this theory, as has been stressed by LAURELL (1951). According to him they suggest instead that the iron leaves the blood stream in ionized form. Transferrin is thus probably a true carrier of iron just as haemoglobin is a carrier of oxygen. The direction of the transport is probably regulated by differences in the ionic concentrations of iron, as stated below.

In healthy human subjects the transferrin is only saturated to about one third of its total iron-binding capacity (TIBC) by the serum iron (LAURELL, 1947). The saturation index, i.e. the serum iron (SI) in per cent of the TIBC, lies at about 33 per cent, and the quotient, SI/UIBC (UIBC = unsaturated iron-binding capacity), at about 0.7. Changes in these relations occur after certain patterns, particularly in iron deficiency states. The typical alterations in this quotient and in the absolute values for SI and TIBC made LAURELL to suppose that the transferrin level is of importance in the mobilization of iron and iron absorption. According to him the hypothesis can be explained by the reversible reaction Fe^{+++} + Fe-free-transferrin $\rightleftarrows$ Fe-transferrin with the corresponding equilibrium equation,

$$\frac{[\text{Fe-transferrin}]}{[\text{Fe-free-transferrin}]} = K \cdot [Fe^{+++}]$$

If this opinion is accepted transferrin should serve as a vehicle for the transport of iron in plasma, and as a regulator of the mobilization of iron from the depots and of the absorption of iron from the cells of the intestinal mucosa. YUILE *et al.* (1950) have shown, however, that in iron-deficient dogs full transferrin saturation, artificially produced by injection of iron salt solutions, did not impair the absorption of radio-active iron. They concluded that the transferrin and its relative saturation played but a little role *per se* in the control of iron absorption. Their results are supported by the observations of SMITH *et al.* (1952), who found that a low and almost completely saturated total iron-binding capacity did not seem to restrain the absorption of iron in nephrotic children excreting large amounts of transferrin and its attached iron.

6. Clinical aspects of serum iron and iron-binding capacity in adults

The serum iron values in healthy adults recorded by different authors have recently been collected by HEMMELER (1951, table 15). Representative investigations on Swedish populations have been made by VAHLQUIST (1941)

TABLE I

	Method	Investigator	Year	TIBC, gamma per cent		
				Men	Women	All cases
Saturation brought about *in vivo*	Serum iron determination on the five-minutes specimen of the 10 mg intravenous tolerance test	BRØCHNER-MORTENSEN	1943	361 (8)	(2)	359 (10)
		WALDENSTRÖM	1944	—	291 (18)	291 (18)
		GREENBERG *et al.*	1947			315 (13)
		TÖTTERMAN	1949	376 (13)	402 (13)	389 (26)
		BRENDSTRUP	1950	(2)	294 (9)	304 (11)
		GITLOW & BEYERS	1952	289 (10)	—	289 (10)
Saturation brought about *in vitro*	'Chemical principle' of HOLMBERG & LAURELL (1945)	HOLMBERG & LAURELL	1945	(6)	(4)	312 (10)
		LAURELL	1947	315 (61)	315 (39)	315 (100)
		DAVIES *et al.*	1952	304 (20)	320 (20)	312 (40)
	'Physical principle' of SCHADE & CAROLINE (1946)	RATH & FINCH	1949	311 (15)	288 (15)	300 (30)
		CARTWRIGHT & WINTROBE	1949	347 (15)	371 (15)	359 (30)
		SMITH *et al.*	1950			364 (11)
		VENTURA & KLOPPER	1951	—	328 (25)	328 (25)
		BRAUNSTEINER *et al.*	1952	343 (17)	352 (8)	—

The total iron-binding capacity (TIBC) in healthy adults according to different authors. Number of es in brackets.

and LAURELL (1947) (100 subjects each). VAHLQUIST found an average of 142 (48—263) gamma per cent in males and 123 (53—210) in females. With a somewhat different method the corresponding values of LAURELL were 124 (70—214) and 108 (57—196). The sex-difference is statistically significant, according to LAURELL. This fact has been put in relation to the smaller fraction of storage iron per kg body-weight in women (LAPICQUE, 1947; ROTH *et al.*, 1951), but the essence of the mechanism is unknown.

As was first stressed by VAHLQUIST (1941) the serum iron shows pronounced diurnal variations with higher morning than evening values in healthy subjects living an ordinary life with a normal diet. This important fact has not been taken into consideration by some workers investigating the normal serum iron. The differences, however, are as large as up to 30 per cent, and must be taken into account in evaluating serum iron levels (VAHLQUIST, 1941; HØYER, 1944; WALDENSTRÖM, 1946; NILSSON, 1947; and others). The lowest values are apparently reached during the periods of greatest activity (HAMILTON *et al.*, 1950). A factor of great importance for the daily variations seems to be the duration and intensity of sleep (HEMMELER, 1944, 1951). After a short and restless night the fasting values in the morning are as low as in the evening, and a total reversal of the diurnal rhythm is found in people working at night

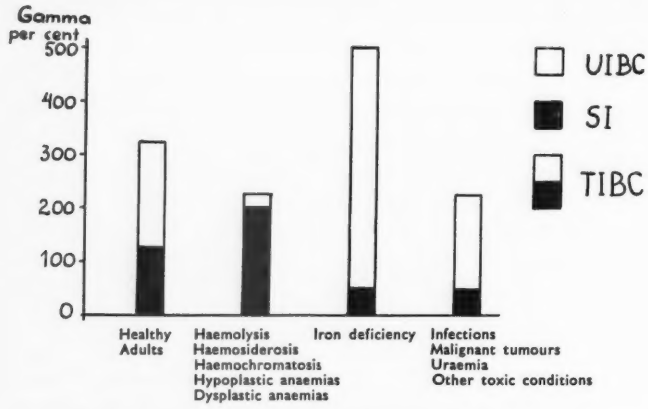

Fig. 1. *Characteristic changes in the serum iron and the iron-binding capacity in conditions with overflowing iron stores, iron deficiency, and toxic states.*

and sleeping during the day (WALDENSTRÖM, 1946). Another factor influencing the diurnal variations appears to be the intake of food, as the serum iron values were found to increase during the day in fasting individuals (JACOT, 1951).

The average TIBC in healthy adults ranges from 300 to 360 gamma per cent. The figures of different authors are quoted in table I and compared with various serum iron values taken 5—15 minutes after a 10 mg intravenous iron tolerance test, i.e. the direct measurement of the TIBC by means of WALDENSTRÖMS 'braking effect' (1944), a technique, however, only valid with special reservations (HAGBERG, 1953a).

Whereas the serum iron level is subject to diurnal variations under normal conditions and rapid fluctuations during iron tolerance tests and in various pathological states, the TIBC appears to be much more stable (LAURELL, 1947, 1952). No immediate changes have been observed after oral or intravenous iron tolerance tests, nor have diurnal variations been found (LAURELL, personal communication; SMITH *et al.*, 1952).

When the iron-balance is disturbed the serum iron level and the iron-binding capacity change according to certain typical patterns (LAURELL, 1947, 1951, 1952; RATH & FINCH, 1949; CARTWRIGHT & WINTROBE, 1949; VENTURA & KLOPPER, 1951; BRAUNSTEINER *et al.*, 1952). For the understanding of the general physiology of iron the following principal changes in states of iron deficiency and of iron excess are fundamental (fig. 1). Pure iron deficiency and most other conditions with increased requirements of iron (e.g. pregnancy and post-haemorrhagic states) are characterized by a low serum iron, increased UIBC and TIBC, and, consequently, a low saturation index and a low SI/

UIBC quotient. On the other hand, conditions with an increased storage of iron (haemosiderosis, haemochromatosis, haemolytic anaemias, and anaemias of dysplastic, hypoplastic, or aplastic origin) are characterized by an increased serum iron, a decreased TIBC, and a high saturation index and quotient. Finally in infections, various intoxications, and malignant tumours, the two factors vary in a specific manner independently of the iron supply; this will be further discussed below.

CHAPTER II

Iron Metabolism in Infancy and Childhood

Interest in the special problems of iron metabolism in the new-born and growing organism dates from BUNGE's early investigations on iron in young mammals (1889—1892). Among other things he and his school (ABDERHALDEN, 1898 and HÄUSSERMANN, 1897) were able to show that the amount of tissue iron in a young rabbit, dog, or cat, was high at birth, remained nearly constant during the first weeks of life, and later diminished. He held that the liver in particular of the new-born animal contained a large store of iron which could be drawn upon during the period of lactation as the milk was found to be a proportionally poor source of iron. In 1902 BUNGE pointed out the importance of his findings in infant feeding, and suggested that infants fed too long on milk would probably become anaemic after exhaustion of the liver store.

Although we now know that the magnitude and the physiological importance of the fetal liver iron stores were overemphasized in infants, and other iron sources underestimated, by these pioneer investigators, the same problems remain of interest. The rapid changes in iron balance during intrauterine and early extrauterine life always make the study of available iron stores a central factor in investigations on iron metabolism in growing organisms.

1. The storage of iron in the fetus and the new-born

The total body iron in a full-term, new-born infant amounts to about 300 mg (HUGOUNENQ, 1899; CAMERER & SÖLDNER, 1900; JERLOV, 1934; IOB & SWANSON, 1938; LINTZEL *et al.*, 1943; WIDDOWSON & SPRAY, 1951), i.e. one and a half times the amount per kg body-weight in adults. Most of the iron is acquired during the last two months of intrauterine life (JERLOV, 1934; SWANSON & IOB, 1939), as can be seen from figure 2. The curves illustrate clearly that a large quantity of iron is lost through premature birth.

During early fetal life the storage iron fraction is mainly formed by an increase in the concentration of tissue iron, later solely by a growth of the storage organs (RAMAGE *et al.*, 1933; IOB & SWANSON, 1938). As far as possible the fetus seems to furnish itself with the iron needed at the expense of the mater-

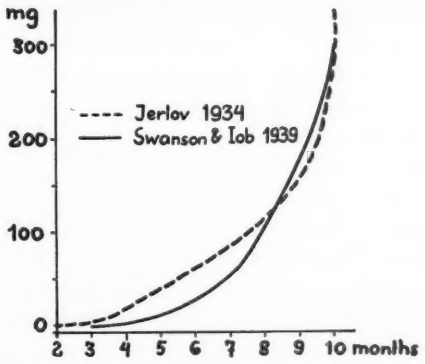

Fig. 2. *The total body iron of the human fetus according to* JERLOV (*1934*), *and* SWANSON & IOB (*1939*).

nal iron stores (JERLOV, 1934). Studies with radio-iron absorption tests (OETTINGER *et al.*, 1949) thus suggest that adequate iron stores are present at birth, regardless of the maternal haemoglobin level. It has further been found (STRAUSS, 1933) that children of mothers with a pronounced iron deficiency anaemia never present any symptoms of iron deficiency at birth though their iron stores are somewhat smaller than the average (TOVERUD, 1935).

In spite of an iron flow favouring the fetus, the fetal depots do not contain excessive amounts of iron at birth. WIDDOWSON & SPRAY (1951) found that the liver contained no more than 30—50 mg of inorganic iron, or some 15 per cent of the total body iron, available for growth. Probably because of difficulties in exact estimation of the storage iron fraction a wide range of average values and individual variations is to be found in the literature (GLADSTONE, 1932; RAMAGE *et al.*, 1933; TOVERUD, 1935; IOB & SWANSON, 1938; BRÜCKMANN & ZONDEK, 1939; LINTZEL *et al.*, 1943; ROTH *et al.*, 1951).

The existence of a continued post-natal iron storage during the first two months of life was first stressed by SCHWARTZ *et al.* (1924), and GLADSTONE (1932). It is caused by a partial redistribution of haemoglobin iron to the depots after the adjustment of the haematopoietic system to extrauterine life following the intrauterine existence poor in oxygen. The importance of the post-natal storage of iron has been pointed out in particular by STEARNS & MCKINLEY (1937), who held that the blood constitutes the main iron store in the baby. They calculated the post-natal stores to be 250—300 mg iron. Even if these values are somewhat too high (MCCANCE & WIDDOWSON, 1951), they nevertheless emphasize the contrast to the 50 mg present in the liver at birth.

The development of the siderosis has been demonstrated by LANGLEY's

(1951) recent histo-chemical investigations which indicate a temporary fall in the visible iron content of the storage organs during the first three days of life, followed by a progressive rise over the next 3—4 weeks. After 2—3 months there is a successive decrease in siderosis. These findings are roughly in agreement with chemical analyses of the iron content of the liver and spleen at various ages (RAMAGE *et al.*, 1933; BRÜCKMANN & ZONDEK, 1939; LINTZEL *et al.*, 1943; WIDDOWSON *et al.*, 1951). Thus the total amount of iron in the storage organs seems to be somewhat larger in the age group ½—2 months than in the new-born. There is then a sharp drop during the second half of the first year. The minimum average values are not reached until the age of 2 years. After that time the values are found to rise slowly again, reaching the adult level at approximately 20 years of age.

In addition to the iron stored during the fetal period, the food is the second source of iron available to the growing infant. Until recently the extent of this supply was unanimously considered to be very small, as the quantity in breast milk averages not more than about 50 gamma per cent (LESNÉ *et al.*, 1930; WALLGREN, 1932; FEUILLEN & PLUMIER, 1952), and is about the same in cow's milk. In 1951, however, MCCANCE & WIDDOWSON asserted that a baby probably increases its total body iron by some 40 per cent during suckling (6 mths.), and that this iron might be obtained from the breast milk. Their findings are, on the other hand, contradicted by the results of studies with radio-iron transplacentally transferred (SMITH *et al.*, 1950), which demonstrate that dietary iron makes only a negligible contribution to the haemoglobin during the six months following birth.

So far it thus seems difficult to say for certain whether blood-iron or milk-iron is the more important factor in maintaining iron-balance during the first half year of life. Be that as it may, the fundamental fact is that even a small restriction in any one of the two sources of iron mentioned probably causes iron deficiency. In other words, the margin of safety seems to be small. Symptoms of iron deficiency during the second half of the first year of life have been described 1) in untreated full term infants whose mothers had an iron deficiency anaemia during pregnancy (STRAUSS, 1933; JERLOV, 1934; MACKAY, 1935), and 2) in infants whose umbilical cords were clamped immediately after birth, depriving the baby of considerable amounts of haemoglobin iron (WILSON *et al.*, 1941; contradictory results have, however, been published by SELANDER, 1945), and 3) in infants with inadequate food or nutritional disturbances (MACKAY, 1931, 1946).

2. Blood morphology in relation to the changes in depot iron

The relative anoxia of intrauterine life requires an increased capacity for the transport of oxygen. The mean haemoglobin concentration of capillary blood at birth is thus as high as 19.5 gram per cent. The red cells are not increased quite as much, amounting to 5.4 million per cu.mm. (VAHLQUIST, 1948). After a temporary rise in the very first days both values gradually decrease during the following eight to ten weeks. During this period the initial macrocytosis is successively supplanted by a slight microcytosis. Hence the 'colour index' diminishes from supernormal to slightly subnormal values.

The fall in the red blood count during this first stage parallels the developing post-natal siderosis in the liver. Both have usually been attributed to excessive haemolysis following birth (for references see KÜNZER's monograph, 1951). There is, however, no convincing evidence of hyperhaemolysis (VAHLQUIST, 1941; FINDLAY *et al.*, 1947; SCHÄFER, 1949; LANGLEY, 1951), nor do survival studies using the Ashby method (MOLLISON, 1951) support an increased erythrocyte break-down of any importance in the new-born. On the other hand, the 'physiological anaemia' of the new-born and the increased content of iron in the liver might well be interpreted as being caused mainly by suppression of the erythropoietic activity of the bone marrow (JOSEPHS, 1932; VAHLQUIST, 1941; SCHÄFER, 1949) together with the post-natal falling off of extramedullary haematopoiesis (LANGLEY, 1951).

The second stage begins about three months after birth and is characterized by an unchanged haemoglobin level and a slightly rising red cell count, indicating increasing microcytosis. These changes in the blood picture corre-

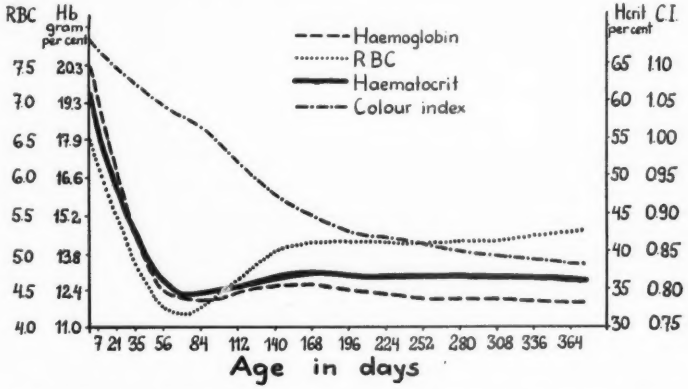

Fig. 3. *Changes in haemoglobin, red cell count (RBC), haematocrit, and colour index (C.I.), in breast-fed infants during the first year of life (according to* HORAN, *1950).*

spond to the stage of decreasing iron storage in the depots. The various alterations in the red blood picture during the first year of life are shown schematically in fig. 3, from the results of a recent investigation by HORAN (1950).

3. Serum iron in early life

The first investigation on normal serum iron values in infancy and childhood was made by THOENES & ASCHAFFENBURG (1934). In spite of a troublesome and somewhat unreliable method, their observations are still to a large extent relevant. In 1941 VAHLQUIST's important monograph was published. In a normal series of about 350 healthy infants and children he studied the various changes in serum iron from the first day of life to puberty. He also estimated the values in fetal blood at different stages of gestation, and in umbilical cord blood from full term infants at delivery. The values given below all originate from VAHLQUIST's investigations (1941 and MÖLLER & VAHLQUIST, 1946). The changes during the first year of life are given in fig. 4.

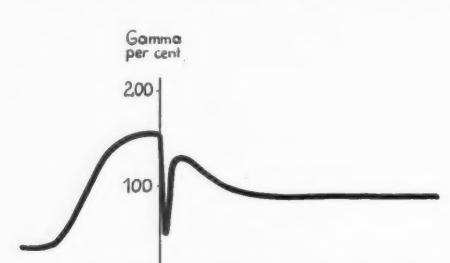

Fig. 4. *The variations in the serum iron during fetal life, at birth and during the first twelve months (after* VAHLQUIST, *1941, revised figures 1946).*

During early fetal life the serum iron level seems to be low. Until the sixth month of gestation values as low as 20—40 gamma per cent can be measured. There follows apparently a successive increase culminating in the strikingly high final level of about 140—160 gamma per cent, reached a few weeks before term. Thus the changes in serum iron during fetal life roughly parallel those of the total body iron and the stored iron.

During the first 24 hours of life there is a sharp drop in the serum iron. The values decrease from the high level of about 160 gamma per cent at birth down to some 50 gamma per cent. During the following two weeks there is again a rise to a level somewhat lower than that of the umbilical cord. The

serum iron values during the remainder of the first two months, i.e. the period of increasing storage of iron, have not been examined separately. VAHLQUIST's 24 cases in the age group 1—6 months show, however, a successive decrease to an average of some 90 gamma per cent (1946, revised values) during the second half year of life. This level is maintained until at least 2—3 years of age. At the age of seven years the mean level has risen again to about 100 gamma per cent. The female adult level is reached by both sexes at pre-puberty, whereas the decided increase in the male values apparently occurs between the ages 15—20 years.

The initial drop in the serum iron on the first day of life seems to be best explained by the cessation of the transfer of iron from the mother. The following successive rise is compatible with the beginning post-natal storage during the hyporegenerative stage of haematopoiesis.

The most debated changes refer to the second fall which takes place gradually after the first 2—3 months of age, the values then remaining stationary over at least the following two years. In connection with the microcytosis and the low content of stored iron it is tempting to assume that children in this age group have a real iron deficiency anaemia. According to VAHLQUIST (1941) the changes in the blood occurring at this time are, however, not an expression of sideropaenia but are physiological manifestations. The interpretation of the various findings and opinions will be further discussed in relation to my own investigations.

As in adults the serum iron level in children is also subject to diurnal variations. These have been studied by VAHLQUIST (1941), SCHÄFER (1949), and MAURER (1952). The same diurnal rhythm as in adults has generally been found, even in infants of less than one year of age (VAHLQUIST, 1946 — see MÖLLER & VAHLQUIST). It seems, however, to be less pronounced during the

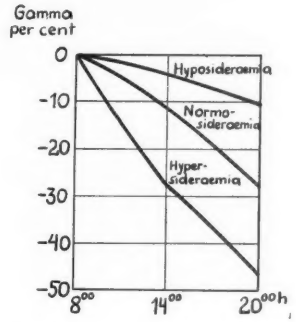

Fig. 5. *Average diurnal lowering of the serum iron in 48 children aged 3—13 years (from* MAURER, *1952).*

first three months of life (VAHLQUIST, personal communication). The degree of the fluctuations seems to be related to the initial fasting level (MAURER, 1952, fig. 5).

4. Earlier investigations on the iron-binding capacity in infancy and childhood

In spite of the interesting fluctuations of the iron balance taking place during the first years of life, the corresponding changes in the TIBC have not yet been satisfactorily established. When this work was started in 1950 only the values in the new-born infant at parturition were known (LAURELL, 1947). To quote LAURELL: 'The mean value for 25 new-born children was 226 ± 10 gamma per cent, with a serum iron concentration of 147 gamma per cent. How rapidly the normal adult value for iron-binding protein is attained is still to be investigated.'

In 1950 SMITH and associates determined the iron-binding capacity in children with Mediterranean anaemia, and also, as a comparison, in 11 healthy children varying in age between 2 weeks and 10 years. Recently SMITH *et al.* (1952) published their investigations on the serum iron and the iron-binding capacity in healthy infants and children and in young patients with various blood disorders. Their normal series consists of 51 subjects, of whom 22 were less than one year of age. Only 7 determinations, however, were made in the important stage 1—6 months, where the available storage iron is altered more than at any time in later life.

The results obtained in the investigation carried out by SMITH and associates will be discussed in relation to my own investigations.

CHAPTER III

Methods

1. Determination of the serum iron

The determinations of the serum iron were made according to the well-established principles first set out by HEILMEYER & PLÖTNER (1937). A micro-modification of VAHLQUIST's method (1941) was used.

The principle of the method is as follows. The protein-bound iron of the serum is liberated after acidification with hydrochloric acid. The proteins are fully precipitated with trichloracetic acid. The protein-free supernatant iron salt solution is adjusted to a suitable pH and the ferric iron reduced with hydroquinone to ferrous iron, which is converted into a coloured iron complex by the addition of ortho-phenanthroline hydrochloride. The intensity of the colour, read spectrophotometrically, is proportional to the iron concentration.

Technique

Cleaning of glassware. All glassware used was treated with potassium dichromate in concentrated sulphuric acid and washed in repeated changes of iron-free water (passed through a cation exchange column).

Reagents and solutions.

6 M hydrochloric acid (pro analysim)
20 % trichloracetic acid (pro analysim)
Concentrated ammonia (pro analysim)
M/2 hydrochloric acid
para-nitrophenol, 1 %
Hydroquinone, 2 %
O-phenanthroline hydrochloride, 1 %

Analysis. To 0.3 ml serum are added 0.3 ml water (iron-free) and 0.3 ml 6 M hydrochloric acid, with constant shaking. After 15 minutes 0.6 ml trichloracetic acid is added, again with shaking. 20 minutes later the mixture is centrifuged for 15 minutes (at 1 100 *g*, Corda). The supernatant is cautiously taken off with the aid of a Pasteur pipette and 1 ml used for the analysis. After the addition of one drop of p-nitrophenol as indicator the mixture is neutralized with concentrated ammonia to the exact point when a yellow colour is first seen. The mixture is then acidified with M/2 hydrochloric acid, 0.03 ml more than necessary to make the colour disappear again. One drop of hydroquinone and one drop of o-phenanthroline hydrochloride are added, and the final volume calculated according to VAHLQUIST. After half an hour or more the extinction value is read against water in a Pulfrich photometer with 50 mm microcuvettes and filter S 50.

Blanks were obtained by using iron-free water instead of serum. They ranged from 14 to 35 gamma per cent, average 24, the variations depending mainly on the purity of the trichloracetic acid used.

The formula for converting the values to gamma per cent can be found in VAHLQUIST's monograph (pp. 73—74).

Error of measurement

On the basis of 100 arbitrarily chosen duplicate determinations the standard error was calculated to $\sigma_m = 6$.[1] This implies that practically no single estimation deviates more than 18 gamma per cent from the real value.

All values recorded in this investigation are the mean of duplicate determinations for greater accuracy, σ_m being $6/\sqrt{2}$ or 4.2. Thus the error of the measurement applied here only amounts to about 4 per cent when the serum iron is 100 gamma per cent.

Comparison with the original method of VAHLQUIST

30 samples were simultaneously analysed by VAHLQUIST's original method and the micro-modification. Each value used for comparison was the mean of duplicate determinations. The figures corresponded well, the correlation coefficient being $+0.98 \pm 0.01$ for samples with serum iron levels between 72 and 175 gamma per cent. In 8 cases identical values were found. The average deviation between the results was 4 gamma per cent and was not systematic. With VAHLQUIST's method the mean value of the group was 113.53 gamma per cent, and with the micro-modification 113.66. As can be seen, the difference is insignificant (0.13 ± 1.1).

Discussion

The method used differs from VAHLQUIST's in the following ways.

1. 0.3 ml serum was used instead of 2 (1) ml.
2. Before acidification the serum was diluted with its own volume of iron-free water.
3. After precipitation of the proteins the sample was centrifuged instead of filtered.

The amount of serum was reduced, partly as a link in the attempt to obtain micro-methods for laboratory analyses in paediatric practice, and partly

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[1] The formula used was $\sigma_m = \sqrt{\frac{\Sigma d^2}{2n}}$ (after DAHLBERG, 1940), where d is the difference between the two values obtained for the same sample, n the number of differences, and σ_m the error.

to try to cut down the size of the blood sample needed for determination of the TIBC. The volume of 0.3 ml serum for the analyses was chosen after preliminary tests with diminishing amounts of serum. It was found to be the least amount which could be safely employed.

With the small amounts of serum used dilution to a larger volume was necessary to bring the final amount to slightly more than 1 ml for spectrophotometric determinations in the microcuvettes of the Pulfrich Stufenphotometer. The disadvantage of the dilution was that the errors of the readings were doubled. On the other hand far fewer opalescent reaction-mixtures following the addition of hydrochloric and trichloracetic acids were noticed.

Centrifuging was also adopted to make a larger amount of reaction-mixture available for the final reading, as quite a lot of the solution is lost during filtration. No inconvenience was suffered from this alteration of the method. On the contrary, one source of error, the possibility of accidentally introducing iron with the filter-paper, was eliminated.

2. Determination of the total iron-binding capacity of serum

At present it is possible to determine the TIBC in three quite different ways. The oldest, originally used by SKOUGE (1939) and WALDENSTRÖM (1944), is to examine the 'five minute specimen of the ten milligram intravenous iron tolerance test'. It has recently been revived by CARTWRIGHT & WINTROBE (1949) and GITLOW & BEYERS (1952), who consider it to be the simplest and most satisfactory way to measure the TIBC. The method of HOLMBERG & LAURELL (1945) and LAURELL (1947) is based on the fact that iron added to serum in excess of the TIBC reacts with $\alpha\alpha_1$-dipyridyl or ortho-phenanthroline in the presence of a small amount of sodium hydrosulphite. The third method is based on the investigations of SCHADE & CAROLINE (1946). They found that progressive development of a salmon-red colour occurs on the addition of ferrous iron to beta$_1$-globulin until the protein becomes saturated. At the point of saturation there is a sharp break in the development of the colour (fig. 6). From the amount of iron needed to reach this point the UIBC may be calculated (RATH & FINCH, 1949; CARTWRIGHT & WINTROBE, 1949). The TIBC is then obtained by adding the figures of the UIBC and the serum iron.

The original intention was to employ the method of LAURELL (1947). The advantages are that 1) it seems to be the most reliable method especially when the UIBC is low; 2) it can be used on any kind of serum without limitation; and 3) it may be carried out on less than 1 ml serum (0.2 ml according to DAVIES *et al.*, 1952), which is of importance when dealing with children.

When this work was started it was, however, impossible to obtain fresh

sodium hydrosulphite *pro analysim*. As intravenous iron tolerance tests in children are unsuitable and unpractical and this method commonly gives unreliable values unless the test dose is closely related to the body-weight (HAGBERG, 1953a), the third method, based on the principles of SCHADE & CAROLINE, was adopted. The modification by RATH & FINCH (1949) was used, as the same type of spectrophotometer was available.

Technique

Solutions required. Standard iron solution containing 20 gamma of ferrous iron per ml, prepared by diluting 14 mg of ferrous ammonium sulphate + 0.5 ml 1 M acetic acid in 100 ml iron-free water.

Physiological saline (iron-free).

Analysis. Serum from fasting subjects was used (see p. 38). The analyses were performed in a Coleman spectrophotometer, Model 14, with square cuvettes (14—307) 1.3 cm deep.

2 ml of serum and 3 ml of saline are pipetted into one of the cuvettes, and 5 ml saline is placed in the other. The transmission for the last cuvette is corrected to 100 per cent and thus serves as a blank. Addition of iron to this cuvette was found to be unnecessary. After 10 minutes the transmission of the serum dilution is measured several times and used as the basic value. Standard iron solution is then added to the sample in quantities of 0.05 ml at a time, and the cuvette rocked to mix thoroughly. Several readings are taken 2 minutes or more after each addition of iron. Before each reading the blank is checked. Fractionated addition of iron is thus made until no further change in the transmission occurs on three successive readings. The difference in transmission is converted into a percentage of the basic value. The figures plotted on graph paper give two straight lines, one representing the decrease in transmission, and the other the constant transmission of the end-point (fig. 6). The point of intersection of these two lines represents the amount of iron added, i.e. the UIBC. The TIBC is obtained by adding the serum iron value.

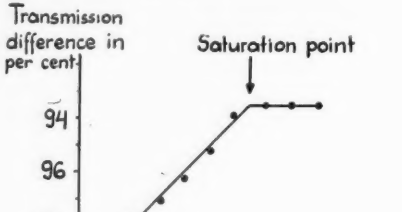

Fig. 6. *A typical iron titration curve.*

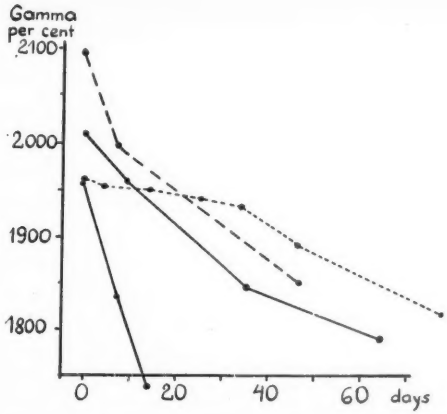

Fig. 7. *The fall of the iron concentration in the standard solution.*

Stability of the standard iron solution

The stability of the solution was checked by repeated determinations of the iron concentration. It was found that this steadily fell, the rate, however, varying greatly from solution to solution. Some examples are given in fig. 7.

The cause of the fall was found to be a deposition of iron on the walls of the vessels. This source of error is of no practical importance when the UIBC is low (50—100 gamma per cent), but when it is raised the error may be considerable. Throughout this investigation, therefore, the fall in the iron concentration of the standard solution has been taken into account.

Error of measurement

The error was calculated[1] from duplicate determinations made on 148 samples representing different levels of the UIBC. The average standard error was found to be 13 (table II), which corresponds to about 5—10 per cent of the values when the UIBC exceeds 100 gamma per cent. As the absolute error is practically constant at all levels of the UIBC the method is far less reliable for levels between 50 and 100 gamma per cent. Values below 50 gamma per cent cannot be determined by the method of RATH & FINCH but may be roughly calculated from the standard curve described below (fig. 8). As the standard iron solution used contains minimal amounts of ferric iron there must be, in addition to the above-mentioned error of the method, a systematic error which, however, is probably inconsiderable. It is due to the fact that

[1] According to the formula $\sigma_m = \sqrt{\frac{\Sigma d^2}{2n}}$ (see footnote p. 26).

TABLE II

UIBC level	No. of blood samples	S. D.	S. D. in per cent of mean UIBC
< 100	19	13	21
100—200	40	14	9
200—300	62	11	5
300—500	27	16	4
All values	148	13	

Determination of the error of the method by duplicate estimations at different levels of the unsaturated iron-binding capacity (UIBC). S. D. = standard deviation.

the transferrin combines at a slower rate with trivalent than divalent iron (SURGENOR *et al.*, 1949), and consequently also takes a longer time to develop the corresponding increase in serum colour.[1]

Checking the method by means of serum iron determinations following iron tolerance tests

Intravenous iron tolerance tests. The total iron-binding capacity (SI + UIBC = TIBC) was initially measured and then compared with the serum iron level of specimens collected 5, 15, and 60 minutes after intravenous iron tolerance tests (SI = TIBC) with an ionized iron solution. Thus two of the principal methods of determining the TIBC were compared.

Apparently healthy student nurses volunteered. The iron was administered in the form of a 4 per cent solution of ferrous sulphate[2] in doses of 0.130—0.145 mg Fe^{++} per kg body-weight. The injections were made slowly over a period of 5—10 minutes. All of the women reacted with flushing, some of them also with coughing or sneezing, 'pins and needles', and slight tachycardia.

Table III demonstrates that the values for the TIBC measured before the injection of iron corresponded in every case with the serum iron levels obtained 5, 15, and 60, minutes after the injection, provided that saturation was complete. In samples where this was not so, i.e. where a residual UIBC could be shown, the TIBC determined before tallied with the TIBC measured after the injection.

Oral tolerance tests. A more physiological way of trying to reach the satura-

[1] WOLFF (Biochem. Ztschr. *322*: 344, 1952), however, has recently found that *both* ferrous and ferric iron solutions when added to serum *in vitro* give an immediate and full development of the Fe-transferrin colour.

[2] made available through the courtesy of AB Pharmacia Ltd., Uppsala.

TABLE III

	mg Fe per kg b.-wt.	Before injection			After injection								
					5 min.			15 min.			60 min.		
		SI	UIBC	TIBC	SI	UIBC	TIBC	SI	UIBC	TIBC	SI	UIBC	TIBC
1	0.144	47	422	*469*	369	83	*452*	333	104	*437*	321	125	*446*
2	0.143	80	215	*295*	334	0	*334*	332	0	*332*	324	0	*324*
3	0.142	139	193	*332*	359	0	*359*	333	0	*333*	324	0	*324*
4	0.142	184	118	*302*	351	0	*351*	324	0	*324*	317	0	*317*
5	0.140	110	216	*326*	330	0	*330*	329	0	*329*	297	0	*297*
6	0.140	59	223	*282*	302	0	*302*	285	0	*285*	282	0	*282*
7	0.139	129	229	*358*	310	0	*310*	343	0	*343*	333	0	*333*
8	0.138	145	164	*309*	252	69	*321*	254	61	*315*	225	86	*311*
9	0.138	59	437	*496*	527	0	*527*	525	0	*525*	467	0	*467*
10	0.135	76	257	*333*	333	0	*333*	334	0	*334*	333	25	*358*
11	0.133	88	246	*334*	324	0	*324*	315	0	*315*	306	0	*306*
12	0.133	133	198	*331*	366	0	*366*	357	0	*357*	351	0	*351*
13	0.131	44	262	*306*	—	—	—	315	0	*315*	276	42	*318*

Determination of the serum iron (SI) and the unsaturated iron-binding capacity (UIBC) before and after intravenous iron tolerance tests using injectable ferrous sulphate.

TABLE IV

	Sex	mg Fe given	Before iron intake			2 hours after			4 hours after			6 hours after		
			SI	UIBC	TIBC	SI	UIBC	TIBC	SI	UIBC	TIBC	SI	UIBC	TIBC
1	♀	280	68	340	*408*	224	194	*418*	432	0	*432*	—	—	—
2	♀	560	108	312	*420*	330	129	*459*	490	0	*490*	498	0	*498*
3	♀	560	88	374	*462*	390	45	*435*	442	0	*442*	430	0	*430*
4	♀	560	126	211	*337*	326	0	*326*	348	0	*348*	354	0	*354*
5	♀	560	117	252	*369*	—	—	—	388	0	*388*	406	0	*406*
6	♂	560	122	225	*347*	304	90	*394*	408	0	*408*	370	0	*370*

Oral iron tolerance tests. The iron tablets used were 'Novofer' (Pharmacia) containing 0.25 g ferrous tartrate and 0.0025 g cupric sulphate.

tion limit is to supply iron by mouth. Owing to the regulation mechanism for the absorption of iron the undamaged intestinal mucosa only permits a small amount of iron to pass into the blood, and thus the saturation limit is never reached in many healthy subjects in spite of large test-doses of iron. As iron salts are better absorbed in iron deficiency states, some of the tolerance tests were carried out on women with slight hypochromic anaemia. Iron was given in the form of 5—15 Novofer tablets (Pharmacia) containing 0.25 g ferrous tartrate (= 56 mg Fe) and 0.0025 g cupric sulphate. Full saturation was only

achieved in six cases out of seventeen. These six cases have been recorded in table IV. Two of them (nos. 1 and 2) developed nausea, blanching, and faintness. The relationship of these symptoms to a possible exceeding of the TIBC has been discussed in another paper (HAGBERG, 1953a).

It is obvious that the figures here do not tally so well as in the intravenous tolerance tests. At four and six hours after ingestion the serum iron shows a tendency to slightly higher levels than the TIBC values before the beginning of the test.

Discussion. Intravenous iron tolerance tests give the 'braking point' only when certain precautions as to the dose and the rate of injection are observed (HAGBERG, 1953a). This is due to the fact that the saturation limit for the complex-binding of iron only seems to be relative to the presence in plasma of further, and then apparently loosely-bound, iron (HOLMBERG & LAURELL, 1945; HAGBERG, 1953a). That the tests performed here really gave the 'braking point' is supported by the consistent agreement of the serum iron levels at full saturation on three separate occasions during the first hour after injection. Thus it can also be concluded from the results obtained that the determinations of the TIBC by the method of RATH & FINCH on *normal* sera gave the level of the braking point, i.e. the total iron-binding capacity (TIBC). The oral iron tolerance tests, however, do not agree quite so well, the cause of which is hard to interpret, but they point in the same direction. A simultaneous comparison with LAURELL's method would have been interesting, but for reasons mentioned previously it has not been possible to do this.

Analysis of the relationship between the magnitude of the unsaturated iron-binding capacity and the deepening of serum colour on saturation *in vitro*

LAURELL (1947) investigated whether the change in serum colour after addition of iron was directly proportional to the UIBC level calculated by his method. He found that a single estimation of the change in serum colour after iron enrichment gave an approximate expression for the UIBC. Low and normal values were, however, found to be very uncertain. He presumed that this finding was due to interfering colour reactions between his iron-ascorbic-acid solution and substances in serum other than the Fe-transferrin.

As many iron titration curves for determining the UIBC have been done during my investigations it was considered to be of both theoretical and practical interest to analyse statistically the deepening of serum colour in relation to the UIBC values measured during step-by-step saturation. Much time would be spared if one single measurement after supersaturation with iron would suffice for routine clinical use.

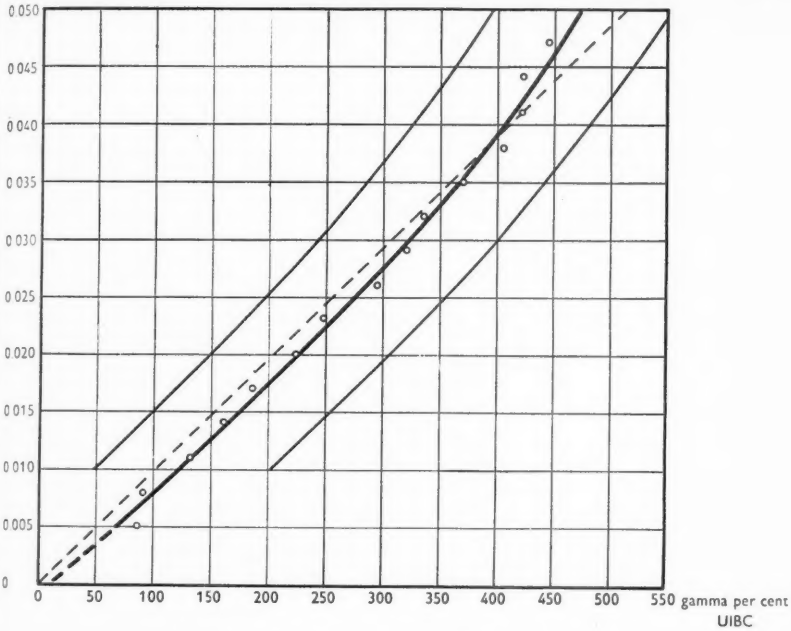

Fig. 8. *The unsaturated iron-binding capacity (UIBC) calculated from the total increase in extinction on titration with iron.* (Continuous thick line = expected mean UIBC for a given increase in extinction. Continuous thin lines = the random variations of the expected mean. Dashed line = Hypothetical relationship between the UIBC and the increase in extinction. This line has been calculated from the average increase in extinction (0.00486) of single median titration steps (UIBC = 50 gamma per cent) and the analysis refers to 177 titration curves).

The total increase in extinction at full saturation and the corresponding UIBC were evaluated in 229 sera (the increase in extinction ranged from 0.004 to 0.048 and the UIBC from 50 to 480 gamma per cent). The values were collected into 15 classes according to the increase in extinction. The class breadth was 0.003. In each class the mean UIBC was calculated, and these mean values are marked as circles in the diagram (fig. 8).

It is obvious that the first twelve mean values fall on a straight line. A linear smoothing of the mean values within the extinction region 0.008—0.035 (by the method of least squares) gives the equation $y = 15 + 10272\ x$[1]. The regression coefficient, 10272, denotes the increase in extinction when the UIBC is increased by one unit. The curve has an *intersept* equal to 15 gamma. This would imply that an average iron enrichment corresponding to 15 gamma per 100 ml is usually necessary to obtain any increase in extinction at all. This finding is supported by practical experience during analysis. Thus it was sometimes found that the first change in serum colour was remarkably small and in a few cases quite failed to appear.

[1] The constructed dashed line (fig. 8) represents the equation $y = 10288\ x$.

TABLE V

Increase in extinction value	Mean value of UIBC gamma per cent	Variations of UIBC (Probability = 0.99)
0.004	54	?
.006	77	?
.008	100	?
.010	123	44—202
.012	145	66—224
.014	166	87—245
.016	187	108—266
.018	208	129—287
.020	228	149—307
.022	247	168—326
.024	267	188—346
.026	285	206—364
.028	304	225—383
.030	322	243—401
.032	339	260—418
.034	356	277—435
.036	372	293—451
.038	388	309—467
.040	404	325—483
.042	419	340—498
.044	434	355—513
.046	448	369—527
.048	461	382—540
.050	475	396—554

Figures referring to fig. 8.

When all 15 mean values are taken into account a parabola may represent the equation[1] slightly better than a straight line. Fig. 8 shows the curve drawn with a continuous, thick line. It expresses the expected mean UIBC for a given increase in extinction. The continuous, thin lines signify the random variations of the UIBC within which in 99 per cent the actual value is to be found. The figures obtained are recorded in table V. High extinction values seemed to give a relatively smaller increase in UIBC. The curve was, however, found to be approximately linear up to extinction values of 0.035—0.040. The surprising deviation above these levels is difficult to explain.

It may be concluded that the 'one stage measurement' of the total extinction increase after enrichment of serum with a supersaturating amount of iron gives far less accurate values for the UIBC than the titration method used in this investigation. The accuracy of the former seems theoretically to

[1] $y = 5.8 + 12.25\,x - 0.0575\,x^2 \pm 31$ y = expected UIBC at ext. $1000\,x$ (method of least squares).

be less than 1/3 of the latter, as step-by-step addition of iron seems to keep the variations within 50 gamma per cent. In practice the differences are probably somewhat less. The 'one stage method' is, however, unreliable and gives only an approximate idea of the true UIBC.

The great variation in the increase in extinction, due to full saturation of various sera at a given UIBC level, supports LAURELL's hypothesis of interfering substances. Moreover, during the first stage of iron titration the increase in serum colour was less than expected. The extent of the change in colour which failed to appear corresponded to that calculated for the addition of about 15 gamma of iron per 100 ml. This finding further stresses that substances interfering with the expected development of the salmon-red Fe-transferrin colour are probably present in normal serum.

Substances in serum interfering with the method

The factor most affecting the accuracy of the method is obviously the presence of certain interfering substances which change the serum colour or make the serum opalescent. What they are is not known, nor is the extent of their role understood (note, however, the observations made above). RATH & FINCH (1949) and CARTWRIGHT & WINTROBE (1949) state that icteric and lipaemic sera cannot be analysed. SMITH *et al.*, on the other hand, found that bilirubin concentrations of 1.0 mg per cent did not interfere with the determinations, and concluded that bilirubinaemia of even high degree had a negligible effect. They did not, however, perform quantitative measurements in different degrees of bilirubinaemia.

As the clinical material reported below was originally intended to include new-born infants in the period of physiological icterus, it was necessary to investigate the quantitative influence of bilirubinaemia. Pure bilirubin (HOMBURGER) was added to serum from a healthy man and a series of serum dilutions of varying bilirubin concentrations made (table VI). The results showed that bilirubinaemia of about 3—4 mg per cent and above interfered with the measurements and gave lowered values. As the bilirubinaemia of week-old babies amounts to 7—8 mg per cent on an average, infants in the first weeks of life were not included in the final series. Older infants with signs of icterus were also excluded. It must be admitted at the same time that serum from the umbilical cord is usually difficult to analyse in spite of bilirubin concentrations below the critical values (1.75 ± 0.11 mg per cent according to VAHLQUIST 1941).

Corresponding investigations were also made on the interfering effect of haemoglobin in serum. It was found (table VII) that even obvious haemolysis did not significantly interfere with the results of the titration method used.

TABLE VI

Bilirubin mg per cent	Degree of visible icterus	UIBC measured gamma per cent	Transmission of light (per cent)
0.6	0	240	73.9
1.1	0	250	69.0
1.6	0	225	66.9
2.1	(+)	230	64.1
2.6	(+)	250	62.0
3.6	+	225	57.9
4.6	+	200	53.5
6.6	+ +	175	48.0
8.6	+ + +	200?	43.0
9.9	+ + + +	125	34.0

The interfering effect of various concentrations of bilirubin on the spectrophotometric measurement of the unsaturated iron-binding capacity (UIBC).

TABLE VII

Haemoglobin added mg per cent	Degree of visible haemolysis	UIBC measured gamma per cent	Transmission of light (per cent)
0.0	0	250	78.0
2.5	0	250	77.7
5.0	0	250	76.1
10	(+)	235	75.2
20	(+)	250	73.0
40	+	250	68.5
60	+	250	64.8
80	+ +	250	60.6
100	+ + +	230	56.9
200	+ + + +	185	39.5

The interfering effect of various degrees of haemolysis on the spectrophotometric measurement of the unsaturated iron-binding capacity (UIBC).

Thus the special precaution of RATH & FINCH, who consistently used syringes coated with mineral oil, could be discarded.

During the investigations it was empirically found that non-fasting sera as well as those from patients with a severely disturbed plasma protein pattern (e.g. a case of disseminated lupus erythematosus) were usually unsuitable owing to turbidity. Other cases with signs of a continuously or temporarily dysfunctioning liver may also have sera which give unsatisfactory curves. Such observations were for instance made in patients with liver cirrhoses, ulcerative colitis, and infectious mononucleosis.

Critical evaluation of the method

Summing up it may be said that this widely used method is far from perfect, but is accurate enough for measurements on normal, clear, fasting sera. It is also valid in various disease states where the serum is not discoloured or lipoid-rich, and where there is no severe disturbance of the plasma protein pattern. These limitations, however, diminish considerably the usefulness of the method, and make it inapplicable in many pathological conditions with changes in the TIBC. Thus the method may falsely suggest full saturation in, for instance, acute hepatitis, where the UIBC is in fact normal or high in spite of an often elevated serum iron level. Haemolytic anaemias and uraemia are other conditions where the method sometimes fails.

It is difficult to assess the accuracy of the method for calculating the TIBC in suitable sera. Presuming that the TIBC is constant during the day and fluctuates only insignificantly in healthy adults from day to day and month to month, the differences between comparable values from the same subject but seldom exceed 50 gamma per cent, and are usually decidedly lower, an opinion supported by the results of the iron tolerance tests.

Estimations on smaller amounts of serum

In order to cut down the amount of serum required for the determination of the TIBC, the reliability of using 1 ml (diluted 1 : 5 instead of 2 : 5) was investigated on 53 samples. Thus analyses were performed both with 2 and 1 ml of the same serum. The results were assessed statistically and found to agree well, the correlation coefficient being $+0.986 \pm 0.004$ (range of UIBC = 50—550). There was no systematic deviation between pairs of values. On an average the results of the determinations using 2 ml serum were 4 ± 3 gamma per cent higher, but this difference is within the limits of random variation. The standard deviation of the differences was found to be 20 gamma per cent.

In spite of this satisfactory agreement the individual 1 ml curves were found to be definitely less reliable as the deviations measured on the spectrophotometer usually became very small. Thus the use of 2 ml was found to be desirable, at least when establishing a normal series, although routine clinical determinations may be performed on 1 ml of serum.

Comparison between venous and capillary blood

VAHLQUIST (1941) has shown that the serum iron levels in venous and capillary blood tally well. As the values presented in this investigation refer to both venous and capillary blood samples, simultaneous analyses of the UIBC on both sorts of blood have been made in 11 cases. The results are recorded in table VIII. They confirm VAHLQUIST's findings on serum iron, and demonstrate that the same good agreement also applies to the UIBC measurements.

TABLE VIII

Sex Age yrs.	SI gamma per cent		UIBC gamma per cent		TIBC gamma per cent	
	venous	capillary	venous	capillary	venous	capillary
♂ 1 6/12	90 (2)	84 (2)	350 (2)	338 (2)	440	422
♂ 4/12	126 (2)	128 (2)	190 (2)	188 (2)	316	316
♂ 6/12	40 (2)	38 (2)	515 (2)	520 (2)	555	558
♂ 28	150 (2)	140 (2)	265 (5)	250 (3)	415	390
♀ 2/12	156 (2)	148 (2)	70	75	226	223
♂ 7/12	—	—	250	244 (2)	—	—
♀ 3/12	—	—	250	250	—	—
♂ 6/12	42	36 (2)	280	280	322	316
♂ 7	—	—	185	185	—	—
♂ 9/12	92	92 (2)	370	341	462	433
♂ 5/12	39[1](2)	34 (2)	300[1](2)	315 (2)	339[1]	349

[1] = arterial

The serum iron (SI) and the unsaturated iron-binding capacity (UIBC) determined on both venous and capillary blood samples taken simultaneously from the same individual. Number of analyses in brackets.

3. Collection and treatment of the blood samples

About 3—4 ml serum is usually needed to perform the determinations. If, however, the UIBC is determined on 1 ml, and single determinations of the serum iron are performed, only 1½—2 ml serum is required. Usually about 10 ml blood was collected for these investigations as duplicate determinations were always made when analysing the serum iron level, and in many cases also when estimating the TIBC. It is often difficult to obtain such large quantities of blood from infants without haemolysis occurring. The following technique was used.

All-glass syringes and chrome nickel needles ('2R2', made in France, manufactured in Sweden by AB Vitrum, Stockholm) were used for the venipunctures, which were performed in a cubital vein when possible, otherwise in the femoral vein just distal to the inguinal ligament.

Capillary blood from the heel was used in infants under one year of age. To obtain such large amounts of capillary blood as 10 ml it is of utmost importance to follow certain precautions. The following procedure was found to be satisfactory.

1. The foot is thoroughly warmed in water of 38—39°C for five minutes, dried, and the heel cleansed with ether.
2. With sharp, newly polished stilettes two moderate punctures are rapidly made close together in the heel.
3. The blood is allowed to drip into an iron-free tube, but not to run down its walls. It is helpful to have the leg hanging down. No other stasis is necessary.
4. Clotting is prevented by frequently wiping the wounds with sterile gauze.
5. Note that the blood must not be squeezed out, as this definitely favours clotting and haemolysis.

TABLE IX

Time in deep-freezer	UIBC measured gamma per cent
4 days	144
10 "	144
19 "	125
2 mths	120
3 "	109
4 "	143
8 "	120
19 "	150

Continuous determinations of the unsaturated iron-binding capacity (UIBC) of a serum kept in a deep-freezer for 19 months.

The samples were left for at least one hour for the clots to retract. They were then centrifuged twice. The serum was pipetted off and frozen down in a deep freezer for analysis days or weeks later.

Preserved in a frozen state at —15 to —20°C, the UIBC seemed to remain unchanged for a long time. An example is given in table IX.

All samples from the clinical material presented in this investigation were treated as above, and the analyses mostly performed within the next two or three weeks.

4. Other haematological methods employed

Haemoglobin was measured with AUTENRIETH-KÖNIGSBERGERS haemometer (standardized according to ENGHOFF), or Haemotest (VEDBAEK) equilibrated with the former.

Erythrocytes were counted according to the usual routine clinical method (ELLERMANN).

Erythrocyte sedimentation rate. The micro-method described by STRÖM (1933) was used.

CHAPTER IV

Clinical Investigations on the Iron-binding Capacity

1. Healthy adults

The serum iron and the total iron-binding capacity (TIBC) were determined in 54 healthy adults in order to obtain a series of basic values for judging the results obtained in infants and children.

Material. The series consisted of 26 men and 28 women, most of them doctors, medical students, nurses, or student nurses. All were in good health without any symptoms of infection during the preceding weeks. The average age of the men was 29 (21—61) and of the women 26 ½ (21—46). The blood samples were collected in the fasting state, between 8 and 9 o'clock in the morning.

Results and conclusions. The results are recorded in table X, and are compared with those of other investigators in table XI.

The serum iron values obtained agree satisfactorily with the findings of other authors, especially with those of VAHLQUIST (1941) who used practically the same technique in his determinations. His corresponding figures were 142 ± 6.1 gamma per cent in 50 men and 123 ± 4.5 gamma per cent in 50 women. The sex-difference was not statistically significant in the present series but the same tendency was found as in larger series.

The values for the TIBC also tally well with the results of other investigators, as can be seen from table XI. The saturation index was, however, some-

TABLE X

	No. of cases	SI gamma per cent		TIBC gamma per cent		SI/TIBC per cent		SI/UIBC	
		M ± ε (M)[1]	S. D.[1]	M ± ε (M)	S. D.	M ± ε (M)	S. D.	M ± ε (M)[1]	S. D.
Men	26	137 ± 6.3	32	321 ± 7.7	39	44 ± 2.6	13	1.0 ± 0.2	0.8
Women	28	123 ± 8.3	44	338 ± 5.9	31	37 ± 2.6	14	0.7 ± 0.1	0.6
Both sexes ..	54	130 ± 5.2	38	330 ± 4.9	36	40 ± 1.9	14	0.9 ± 0.1	0.7

[1] M ± ε (M) denotes mean ± standard error of the mean. S. D. signifies the standard deviation.

The serum iron (SI) and total iron-binding capacity (TIBC) in healthy adults (fasting values).

TABLE XI

Investigator	Year	No. of cases		SI gamma per cent			TIBC gamma per cent				SI/TIBC per cent
		Men	Women	Men	Women	All cases	Men	Women	All cases		
									M ± ε (M)	S. D.	
LAURELL	1947	61	39	124	108	117	315	315	*315 ± 3.3*	33	38
RATH & FINCH....	1949	15	15	106	94	100	311	288	*300 ± 9.4*	51	34
HARTWRIGHT & WINTROBE	1949	15	15	127	123	125	347	371	*359 ± 5.6*	31	35
SMITH *et al.*	1950	11		—	—	163	11		*364 ± 15.4*	51	45
VENTURA & KLOPPER.....	1951	—	25	—	111	—	—	328	*328 ± 9.8*	49	34
DAVIES *et al.*	1952	20	20	126	104	115	304	320	*312 ± 7.5*	47	37
HÖGBERG	1953	26	28	137	123	130	321	338	*330 ± 4.9*	36	40

The serum iron (SI) and the total iron-binding capacity (TIBC) in healthy adults according to various tors.

what higher, possibly indicating that the mean TIBC obtained might have been somewhat lower if the serum iron technique of these other investigators had been used. A small sex-difference in the TIBC, saturation index, and quotient, was found, but it was not statistically significant.

Summing up, the figures obtained in healthy adults agree with the results of other observers, and thus afford a good basis for evaluating the values obtained in infants and children.

2. Mother and child at delivery

The haemoglobin and the red cell count diminish successively during pregnancy. At the same time there is an increase in the plasma volume amounting to 25—50 per cent (e.g. CATON *et al.*, 1949; BUCHT, 1951). In spite of the falling blood values there is, however, an increase in the red cell mass (CATON *et al.*, 1951; LUND, 1951) which corresponds to haemoglobin containing about 500 mg of iron (RATH *et al.*, 1949). As the average loss to the fetus is 300 mg and more iron is needed for building up the placenta and the growing uterus, the extra demand of iron during the whole pregnancy seems to be at least 1000 mg. Although most of the lowering of the blood values is probably explained by 'dilution', it is no wonder that, in addition, signs of iron deficiency may be and have been found during late normal pregnancy (LUNDSTRÖM, 1950), apparently indicating exhaustion of the available iron stores. This assumption is confirmed *ex iuvantibus*, as the symptoms of iron deficiency can be prevented by prophylactic administration of iron by mouth (LUNDSTRÖM, 1950; BEN-

STEAD & THEOBALD, 1952) or saccharated iron oxide intravenously (HAGBERG & LUNDSTRÖM, 1953).

Whereas a negative iron balance is evidently characteristic for the mother, both full term and premature infants have adequate iron stores at the time of birth, regardless of the maternal haemoglobin level. This has already been discussed at some length in chapter II.

Material. The original series consisted of 23 healthy mothers and their infants at delivery. Samples were not obtained in time from two of the women who therefore were excluded. The final, statistically treated series thus consisted of 21 women aged 16—43 years (average 26) and 23 infants with a birth weight varying between 2840—3870 g. In 4/5 of the series the pregnancy was the first or second. The sample from the mother was collected just as the child was born. The values for the infants refer to samples from the umbilical cord taken immediately after it was cut. The blood was allowed to drip freely into the iron-free test tube from the placental part of the cord.

Results. The results are recorded in table XII. The striking differences between mother and child, previously observed by other investigators, were confirmed.

TABLE XII

	No. of cases	SI gamma per cent M ± ε (M)	TIBC gamma per cent M ± ε (M)	SI/TIBC per cent	SI/UIBC
Mother	21	98 ± 6.7	470 ± 15.3	21	0.3
Umbil. cord.....	23	173 ± 6.9	259 ± 10.5	70	2.1[1]

[1] 4 cases excluded where UIBC = 0. Median 2.0 (all cases).

The serum iron (SI) and the total iron-binding capacity (TIBC) in mother and child at parturition.

The average serum iron of the mothers was low but the difference from that of non-pregnant healthy women was not statistically significant. The TIBC, on the other hand, was significantly increased, and the saturation index and quotient significantly lowered, in comparison with non-pregnant values.

The serum iron level of the umbilical cord blood was in contrast significantly raised, whereas the TIBC was far below the average values of healthy adults. This difference was also statistically significant. The saturation index and quotient were the highest measured in healthy human subjects.

Discussion and conclusions. The serum iron levels of the mothers are high compared with the findings of VAHLQUIST (table XIII), but agree somewhat better with LAURELL's figures (table XIII) and those of SUNDELIN (1942) and LUNDSTRÖM (1950). The discrepancies cannot be satisfactorily explained.

TABLE XIII

Investigator	Mother			Umbil. cord		
	No. of cases	SI	TIBC M±ε (M)	No. of cases	SI	TIBC M±ε (M)
VAHLQUIST 1941	30	59	—	32	160	—
LAURELL 1947	SI 17 TIBC 25	80	446±13	SI 20 TIBC 25	147	226±10
HAGBERG 1953	21	98	470±15.3	23	173	259±10.5

The serum iron (SI) and the total iron-binding capacity (TIBC) in mother and child at parturition, a comparison with the results of other investigators.

TABLE XIV

Investigator	Time for sampling	No. of cases	TIBC gamma per cent
LAURELL 1947	P[2]	25	446
FAY *et al.* 1949	40th w.	9	583
RATH *et al.* 1950	P	21	354
VENTURA & KLOPPER 1951	30th—40th w.	25	453
MORGENTHAU *et al.*[1] 1952	P	48	>500
HAGBERG 1953	P	21	470

[1] See SMITH *et al.*, 1952. [2] P = at parturition.

The serum iron (SI) and the total iron-binding capacity (TIBC) in pregnant women at full term.

The finding of a markedly increased TIBC is consistent with the results of all investigators except RATH *et al.* (table XIV). They found strikingly low average values. Their series, however, also shows a tendency towards increased values during late pregnancy. The quantitative differences in TIBC vary greatly from one series to another. To some extent this fact may be explained by the limitations of methods based on the principles of SCHADE & CAROLINE (see chapter III). With an UIBC of the magnitude measured here even small deviations in the iron concentration of the test solution play a significant role. My values agree satisfactorily with those of LAURELL, who used a more accurate method.

Although the average TIBC in pregnant women at delivery is similar to that found in iron deficiency anaemia, it is questionable whether the raised level alone is a sign of iron deficiency; at least when combined with a normal serum iron level such an assumption is unjustified. It is interesting to note that prophylactic administration of intravenous iron (500—1 000 mg) to healthy,

non-anaemic, pregnant women failed to depress the TIBC at delivery (HOLLY, 1951; HAGBERG & LUNDSTRÖM, 1953). The increased TIBC seems therefore to be better explained as an expression of a regulation mechanism directing the iron to the fetus. In contrast to the apparently uninfluenced TIBC, low serum iron values were restored to normal on supplementing the oral iron (LUNDSTRÖM, 1950), indicating recovery from an iron deficiency state.

The serum iron and the TIBC of cord blood agree with the findings of VAHLQUIST (1941), LAURELL (1947), and SMITH *et al.* (1952). Some of the individual TIBC values were, however, approximate, as cord blood often contains large amounts of coloured substances (see p. 35).

Because of the high saturation index and quotient of infants at birth it is tempting to draw a parallel with conditions in which there are excessive iron stores, e.g. haemochromatosis, haemosiderosis, or haemolytic anaemias. The iron stores of the new-born are, however, not at all as unproportionally large (MCCANCE & WIDDOWSON, 1951) as was formerly believed, nor do the low TIBC and high saturation necessarily indicate excessively filled iron stores. They merely seem to signify that iron stores are being built up, i.e. that iron is being diverted from the fetal circulation to the depots. Thus all changes in both mother and child seem to promote a transfer of iron from the depots of the mother to the tissues of the fetus. This explains why even children of grossly iron deficient mothers do not show any signs of sideropaenia at birth (STRAUSS, 1933).

3. Healthy infants and children of all ages

It was previously stressed (chapter II) that the amount of available storage iron apparently changes more during infancy than at any other age. The investigations on healthy infants and children were therefore concentrated to the lowest age groups in order to establish the values corresponding to these rapid fluctuations in the metabolism of iron.

Material. The material consisted of 128 samples from 121 full term infants and children up to 3 years of age. Not less than 78 samples represented ages under six months. Most of the infants were patients from the Paediatric Clinic, Uppsala, chiefly healthy children of sick mothers, and social cases. A few of them were from a babies' home in the same city. Only infants whose red blood count was within the normal limits established by VAHLQUIST (1948) were included. No distinction was made between breast-fed and bottle-fed infants. Children who showed any signs of infection during the preceding week, or were suffering from diseases of the blood, were excluded. All samples were taken in the fasting state between 8 and 9 o'clock in the morning. In the age groups below one year all values refer to 'capillary' blood; in older children

TABLE XV

Age group	No. of cases	SI gamma per cent		TIBC gamma per cent		SI/TIBC per cent		SI/UIBC	
		M ± ε (M)[1]	S. D.	M ± ε (M)	S. D.	M ± ε (M)	S. D.	M ± ε (M)	S. D.
w-born (umbil. cord)	23	173 ± 6.9	33	259 ± 10.5	50	70 ± 4.9	23	2.1 ± 0.4[2]	1.6
-2 mths.	26	142 ± 7.1	36	212 ± 6.6	34	69 ± 4.0	21	2.5 ± 0.4[3]	1.9
-4 "	32	113 ± 5.6	31	308 ± 11.3	64	39 ± 2.5	14	0.7 ± 0.1	0.5
-6 "	20	78 ± 6.1	27	360 ± 12.5	56	22 ± 1.9	9	0.3 ± 0.03	0.2
-12 "	26	93 ± 6.5	33	394 ± 13.4	68	27 ± 2.4	12	0.3 ± 0.03	0.2
-3 yrs.	24	99 ± 5.9	29	387 ± 9.7	47	26 ± 1.6	8	0.4 ± 0.03	0.2
-7 "	21	124 ± 8.7	40	368 ± 9.6	43	34 ± 2.4	11	0.6 ± 0.06	0.3
-14 "	28	119 ± 6.7	35	353 ± 7.6	40	34 ± 2.0	10	0.6 ± 0.05	0.3
ults (both sexes) ...	54	130 ± 5.2	38	330 ± 4.9	36	40 ± 1.9	14	0.9 ± 0.1	0.7

Mean ± standard error. S. D. = standard deviation.
4 cases excluded where UIBC = 0. Median 2.0 (23 cases).
4 cases excluded where UIBC = 0. Median 2.5 (26 cases).
e serum iron (SI) and the total iron-binding capacity (TIBC) at different ages during infancy and lood.

the venous blood was taken from the cubital or femoral veins. With two exceptions sera from infants less than 15 days old were not included as they were usually found to be unsuitable for determination of the UIBC, owing to the presence in serum of large amounts of interfering substances during the period of physiological icterus (see p. 35).

The rest of the material consisted of 49 single samples from non-infected children representing all ages between 3 and 14 years. Most of the children were attending the Paediatric Clinic or the Child Psychiatric Clinic, and the others came from a children's home. Venous blood was collected as described above. Only cases with a normal blood picture have been included.

The whole series was divided into different age groups (see table XV) and evaluated statistically. No distinction was made between umbilical cord blood, 'capillary' blood, and venous blood.

Results. The results are recorded in table XV and sketched in figure 9.

The serum iron level fell from a maximum value at birth to a minimum before the end of the first half year. The difference between the level at birth and at the age of ½—2 months was statistically significant (31 ± 9.9), as was the difference between the latter group and infants of 4—6 months of age (64 ± 9.4). After this time the serum iron remained on a low level for the remainder of the first 2—3 years, showing only a slight increase if any; later it increased more rapidly. Thus the values in children of 3—7 years of age seemed to be higher (difference 25 ± 10.5) than those in the age group 1—3 years, and even agreed with the values in adult women.

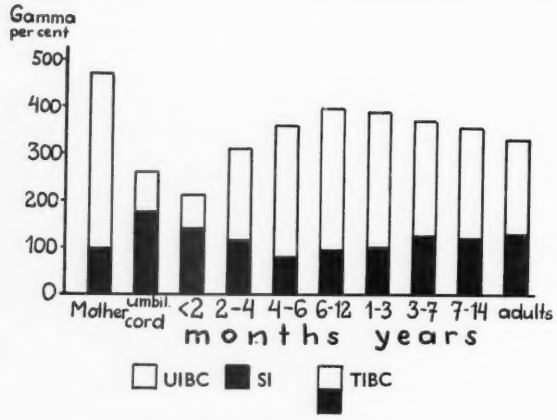

Fig. 9. *The normal series drawn schematically.*

The TIBC, already low at birth, fell further during the first two months. The difference between this and the figures for cord blood is statistically significant (difference 47 ± 12.4). After this initial lowering the TIBC rapidly rose, the increase being statistically significant by 2—4 months (difference 96 ± 13.1). The peak was reached in the age group 6—12 months, with an average value of 394 gamma per cent. This high level was found to be maintained during the next two years, when a successive fall to the adult level appeared to begin. The TIBC, however, seemed to return to normal more slowly than the serum iron. Thus the serum iron of the age group 3—14 years did not differ from the adult level, whereas the corresponding TIBC was significantly higher (difference 29 ± 7.7).

Apart from the first two months of life the TIBC followed the expected pattern, i.e. increased when the serum iron decreased. The maximum changes did not, however, correspond exactly in time, the lowest serum iron value appearing some months before the highest level of the TIBC.

As the serum iron and the TIBC fell relatively uniformly from the levels at birth to those at ½—2 months of age, the saturation index and quotient remained practically unchanged. Thus the high transferrin saturation at birth was found to continue during the first two months of life. After this time the values fell steadily from the highest ever found in healthy human beings to the lowest. The minimum values were already reached at 2—4 months, and then continued on the same low level for the rest of the first two years. The increase which followed seemed to proceed successively until adulthood.

A few infants were examined more than once during the first six months

TABLE XVI

Sex Record, no.	Age, mths.	SI gamma per cent	TIBC gamma per cent	SI/TIBC per cent
♂ 658/52	1 1/4	140	240	58
	2	122	320	38
	3	78	420	19
	4	69	405	17
	4 3/4	37	405	9
♀ 107/52	1 1/2	179	280	64
	1 3/4	136	265	51
	3	134	405	33
	5 1/2	71	385	18
♀ 725/51	1	214	240	89
	2	104	250	42
	3	112	335	34
♀ 745/51	1	146	230	64
	3	108	355	31
♀ 348/51	1 3/4	188	190	100
	3	125	270	47
♀ 1600/51[1]	umbil. c.	138	225	61
	3	88	345	26
♀ 1591/51[1]	umbil. c.	130	280	47
	3	84	370	23
♂ 1535/51[1]	umbil. c.	158	285	55
	3	68	460	15

[1] Record number in the Obstetrical Department.

Repeated determinations of the serum iron (SI) and the total iron-binding capacity (TIBC) in infants during the first months of life.

of life, and these repeated analyses are given in table XVI. They confirm the results of the statistically analysed series.

The sex-differences at various ages were found to be inconsiderable. All results in the ages 3—14 years were evaluated statistically (table XVII). There was a tendency to an elevated TIBC in girls but no statistically significant differences were obtained. The higher values mentioned seemed to be due to random variation.

TABLE XVII

Group	No. of cases	SI gamma per cent M±ε (M)	TIBC gamma per cent M±ε (M)	SI/TIBC per cent M±ε (M)
Children, 3—14 years:				
Boys	25	118± 6.0	349± 9.1	35±2.1
Girls	24	124± 8.9	370± 7.3	34±2.2
Difference		6±10.7	21±11.7	1±3.0
Adults:				
Men	26	137± 6.3	321± 7.7	44±2.6
Women	28	123± 8.3	338± 5.9	37±2.6
Difference		14±10.4	17± 9.7	7±3.6

The sex-differences in children and adults.

TABLE XVIII

Hb gram per cent	No. of cases	SI gamma per cent M±ε (M)	TIBC gamma per cent M±ε (M)	SI/TIBC per cent M±ε (M)	SI/UIBC M±ε (M)
<11.5	29	87±4.7	403±10.6	22±1.2	0.29±0.2
11.5 and over	20	111±6.7	373±13.1	30±1.9	0.45±0.04
Difference		24±8.2	30±16.8	8±2.2	0.16±0.05

The serum iron (SI) and the total iron-binding capacity (TIBC) related to the haemoglobin level in 49 children aged between 6 months and 3 years.

The possible presence of slight iron deficiency anaemias in the series was checked. Children in the age groups with diminished iron stores (6 months—3 years) were divided into two sub-groups according to the haemoglobin level (table XVIII). It was found that those with haemoglobin values less than 11.5 gram per 100 ml had significantly lower serum iron values, saturation index, and quotient. The TIBC, however, did not differ significantly between the two sub-groups. Occasional infants among those with lower haemoglobin levels were found to have values quite in accordance with those found in iron deficiency anaemia.

Discussion and conclusions. The general tendency of the change in serum iron at different ages is in keeping with the findings of VAHLQUIST (1941), and there is also conformity with his corresponding figures for separate age groups (table XIX), if the diurnal variations are taken into consideration. Most of his observations in the ages six months and over refer to samples taken during the afternoon (VAHLQUIST, personal communication). The revised figures of MÖLLER & VAHLQUIST (1946) show that the corresponding morning values

TABLE XIX

VAHLQUIST 1941			SMITH *et al.* 1952			HAGBERG 1953		
Age group	No. of cases	SI gamma per cent	Age group	No. of cases	SI gamma per cent	Age group	No. of cases	SI gamma per cent
14 days	21	125	—	—	—	1/2—2 mths.	26	142
1— 6 mths.	24	89	1— 6 mths.	7	132	2— 6 mths.	52	99
6—12 mths.	23(25)	61[1](87)[2]	6—12 mths.	7	106	6—12 mths.	26	93
1—2 1/4 yrs.	39	58[1]	1— 2 yrs.	7	95	1— 3 yrs.	24	99
7 yrs.	60	103	2— 6 yrs.	9	116	3— 7 yrs.	21	124
11 yrs.	50	109	6—12 yrs.	13	127	7—12 yrs.	28	119

[1] Refers to samples taken during the afternoon.

[2] Revised morning values (MÖLLER & VAHLQUIST, 1946).

The serum iron values (SI) at various ages compared with the results of VAHLQUIST (1941) and SMITH *et al.* (1952).

lie on a level 20—25 gamma per cent higher. My figures for older children also tally with the findings of THOENES & ASCHAFFENBURG (1934), SCHÄFER (1940), and SMITH *et al.* (1952). These authors all give average values of about 120 gamma per cent.

Infants 1/2—2 months. This age group, covering the period of physiologically suppressed erythropoiesis and post-natal siderosis, has not previously been separately investigated with regard to the serum iron level. Although this is significantly diminished in comparison with the findings at birth, it must be noted that the level is still higher than at any other age in life. The TIBC, moreover, shows a significant fall, not formerly known. In this way the saturation index and quotient remain as high as at birth, and thus the same ratio between the serum iron and the TIBC was found at two distinct periods, both with continuous storing of iron going on, although the actual values were different. This clearly supports the hypothesis of LAURELL concerning the regulation of the plasma transport of iron.

Infants 2—6 months. During this period there is a successive return of full haematopoietic activity and simultaneously an increased need for iron in the bone marrow. The direction of the plasma iron transport turns from storage to mobilization. At the same time the serum iron falls to its lowest values, and the TIBC successively rises and passes normal adult levels. The saturation index and quotient rapidly reach minimum values close to those found in iron deficiency anaemia.

Infants and children 6 months—3 years. Due to continuous mobilization of iron during this stage the reserves become more or less depleted. Infants and children of this age are therefore particularly vulnerable to even the smallest

additional strain on the iron balance. Thus simple nutritional iron deficiency anaemias are commoner during this period than at any other age. Even in healthy children there are trends well compatible with a slight iron deficiency anaemia. The microcytosis, the low haemoglobin, and the lowered serum iron levels, in particular have been mentioned.

The problem has been studied *ex iuvantibus* by MÖLLER & VAHLQUIST (1946) and HORAN (1950). Each administered prophylactic doses of iron to healthy infants for six weeks and three months respectively. MÖLLER & VAHLQUIST examined the children at 6—8 months of age and found no difference between the treated group and the controls, either in the red blood picture or in the serum iron level. They concluded that the lower physiological limits are difficult to fix but that the following average blood values are quite physiological for healthy infants after the first half year of life: haemoglobin 12—12.5 gram per cent, red cells 4.5 million, colour index 0.85, serum iron 85 gamma per cent. HORAN made her examinations when the children were 12 months of age. In spite of small differences her findings suggest on the other hand that increasing iron intake influences the haemoglobin, red cell count, and haematocrit reading.

SMITH and associates (1952) investigated the alterations in serum iron and TIBC at certain ages (table XX) and found the same tendency as VAHLQUIST (1941) and myself, i.e. a lowered serum iron level and a markedly elevated TIBC. However, the successive rise to a peak soon after the first half year of life did not appear in their results, probably because of their smaller series and fewer age groups. These authors stress that their findings in infants of 6 months—2 years of age suggest an iron deficiency state despite the absence of anaemia.

My findings (table XVIII) of significantly lowered values for serum iron, saturation index, and quotient, in children with low but normal haemoglobin

TABLE XX

SMITH *et al.* 1952			HAGBERG 1953		
Age group	No. of cases	TIBC	Age group	No. of cases	TIBC
5— 8 days	8	262	½— 2 mths	26	212
1— 6 mths	7	412	2— 6 mths	52	328
6—12 mths	7	429	6—12 mths	26	394
1— 2 yrs	7	414	1— 3 yrs	24	387
2— 6 yrs	9	395	3— 7 yrs	21	368
6—12 yrs	13	340	7—12 yrs	28	353

The total iron-binding capacity (TIBC) in healthy infants and children, a comparison with the values of SMITH *et al.* (1952).

levels suggest that some cases with iron deficiency anaemia are included in the series. This is supported by the results in single cases. These facts indicate that the lower limit of normal haemoglobin values is difficult to fix and probably varies from case to case.

The lowered serum iron, the elevated TIBC, and the decreased saturation index and quotient, imply a heightened ability of the organism to mobilize iron. The comparatively small iron reserves which are characteristic of and probably normal in these ages may explain the need for such a measure. As depleted iron stores are replenished very slowly in adults even on adequate iron therapy (HASKINS *et al.*, 1952), it seems reasonable to suppose that this process will be still more lengthy in growing subjects, and that prophylactic doses of iron by mouth might not suffice either to restore or to maintain the iron depots of a rapidly growing child. Such a mechanism might well explain the failure to restore the serum iron level in series such as that of MÖLLER & VAHLQUIST, and is in keeping with VAHLQUIST's assertion (1941) that a lowered serum iron level must be regarded as physiological in the ages in question.

Children over three years of age. After 2—3 years the iron balance becomes successively stabilized, and the stores replenished through an adequate diet. At the same time the serum iron and the TIBC approach the adult values. However, the TIBC is still significantly higher than the normal adult level, which implies a lower saturation index and quotient. This may even here be explained by the extra demand for iron to provide for growth. JASIŃSKI (1951) also believes in a high frequency (50 per cent) of slight iron deficiency anaemias. He interpreted elevated oral iron tolerance curves as evidence of a real lack of tissue iron even in cases with a normal or high serum iron level. Such a generalisation would appear somewhat sweeping, as uncomplicated iron deficiency states with high serum iron levels do not exist as far as is known. Because of a more labile and vulnerable iron balance all transition forms are, on the other hand, probably common in children, from a physiologically increased demand and a latent iron deficiency state to a slight but obvious iron deficiency anaemia.

Summing up it may be said that the changes in the TIBC during infancy and childhood strongly support the assumption that the transferrin level is of importance in the storage and mobilization of iron. Thus there is a close parallelism between iron storage and low TIBC, and between depletion of the depots and elevated TIBC. There would also usually appear to be a mutual relationship between the transferrin level and the magnitude of the iron stores, but the chief determining factor seems to be the direction of the iron flow.

4. Iron deficiency anaemia

Iron deficiency anaemia is much commoner during late infancy and early childhood than during any other age period. This fact is apparently due to the small stores of iron mentioned above, which do not sustain even apparently slight stresses. The common direct causes of iron deficiency are rapid growth, inadequate and monotonous gruel diet, or ordinary anorrhexia. Predisposing factors are prematurity, twin birth, early clamping of the umbilical cord, and severe iron deficiency in the mother.

In older children severe iron deficiency anaemia does occur (VAHLQUIST, 1941), but in Scandinavian countries it is nowadays rare. Slight forms are commoner, and are usually often connected with abnormal eating habits or postinfectious anorrhexia. Nutritional factors are probably also important causes of the high frequency of iron deficiency anaemia among mentally deficient children (HAGBERG, 1953b).

Infants and children representing the various categories mentioned above

TABLE XXI

Record no.	Sex Age yrs.	Diagnosis	Hb gram per cent	Colour index	SI gamma per cent	TIBC gamma per cent	SI/TI per c
424/52	♂ 1	nutritive iron deficiency anaemia	7.2	0.48	21	475	
161/51	♂ 1½	nutritive iron deficiency anaemia	7.7	0.77	40	475	
52/52	♂ 1	nutritive iron deficiency anaemia + spastic colitis	9.4	0.89	76	500	1
404/51	♀ 1	praematurity + iron deficiency and megaloblastic anaemia	8.0	0.65	64	485	1
820/52	♂ 1	iron deficiency and megaloblastic anaemia	6.5	0.73	42	530	
637/51	♂ 8	idiopathic thrombocytopaenia + hypoproteinaemia + iron deficiency anaemia	9.7	0.81	45	440	1
254/51	♀ 13	iron deficiency anaemia (essential hereditary)	6.1	0.48	31	515	
—	♂ 15	iron deficiency anaemia (essential)	5.1	0.55	34	555	
572/51	♂ 2	cœliac disease + iron deficiency anaemia	10.0	0.84	28	465	
851/52	♀ 11	ulcerous colitis + iron deficiency anaemia	5.4	0.53	25	500	
858/52	♂ 1	iron deficiency anaemia + idiocy	7.0	0.47	42	500	
67/52	♀ 2	" " " + "	8.3	0.61	40	515	
64/52	♂ 4	" " " + "	8.1	0.64	44	500	
201/51	♂ 5	" " " + "	7.5	0.70	78	510	1
512/51	♂ 5	" " " + oligophrenia .	9.4	0.71	117!	460	2
200/51	♂ 6	" " " + idiocy	10.0	0.73	80	500	1

Infants and children with iron deficiency anaemia.

TABLE XXII

Age	No. of cases	SI gamma per cent M±ε (M)	TIBC gamma per cent M±ε (M)	SI/TIBC per cent M±ε (M)	SI/UIBC M±ε (M)
<5 years	9	44	494	9	0.10
5 and over	7	59	498	12	0.14
All ages	16	50±6.5	496±7.1	10±1.4	0.12±0.02

The serum iron (SI) and the total iron-binding capacity (TIBC) in children with iron deficiency anaemia.

have been investigated with regard to changes in the serum iron and the TIBC, and are reported below.

Material. The series consisted of 16 cases (see table XXI), all except one being patients in the paediatric wards. They were aged between 1 and 15 years, and all were free from infection.

Results. Most of the cases had a low serum iron, and all of them a high TIBC and a decreased saturation index and quotient. The lowest TIBC (440 gamma per cent) was found in a case with simultaneous hypoproteinaemia. There was no definite correlation between the degree of the changes in TIBC and the fall in haemoglobin level.

Table XXII shows the statistical evaluation of the series. All values differ significantly from those of healthy children in all age groups. The changes seemed to be of the same magnitude independent of age.

Discussion and conclusions. The results agree perfectly with the findings of SMITH *et al.* (1952) in a similar series. These authors concluded that the pattern of low serum iron and elevated UIBC represents depletion of the iron stores irrespective of a normal or anaemic red blood picture.

Determinations of both the serum iron and the TIBC give a more accurate idea of the need for iron in the tissue stores. It is, however, important to stress that ***both*** values are changed in a true iron deficiency state. This fact is of particular importance in children aged 6 months to 3 years, as healthy children with normal serum iron sometimes have a TIBC quite in keeping with values found in iron deficiency anaemia (table XXIII). Iron therapy would, however, always seem to be indicated when the TIBC is elevated, as this alteration apparently expresses a heightened readiness to mobilize and absorb iron. The presence, but not the extent, of a true iron deficiency is, however, revealed by a raised TIBC only when combined with a low serum iron.

Replenishment of the iron stores is characterized by a successively rising serum iron level and a falling TIBC (fig. 10). The return to normal does not seem to be complete until the haemoglobin is also fully restored. Supernormal

TABLE XXIII

Record no.	Sex Age mths.	Hb gram per cent	Colour index	SI gamma per cent	TIBC gamma per cent	SI/TIBC per cent
966/51	♀ 8	11.8	1.15	106	525	20
988/52	♀ 18	11.2	1.05	140	490	29
759/51	♀ 11	11.5	0.97	206	475	43
(P. B.)	♂ 12	11.1	0.82	94	475	20
(B. S.)	♂ 8	10.9	0.85	99	470	21
835/52	♂ 8	11.1	0.98	92	450	20

Healthy infants and children aged 6 months—3 years with a total iron-binding capacity (TIBC) over 450 gamma per cent in spite of normal haemoglobin levels, and serum iron values (SI) on or over the average level for the age.

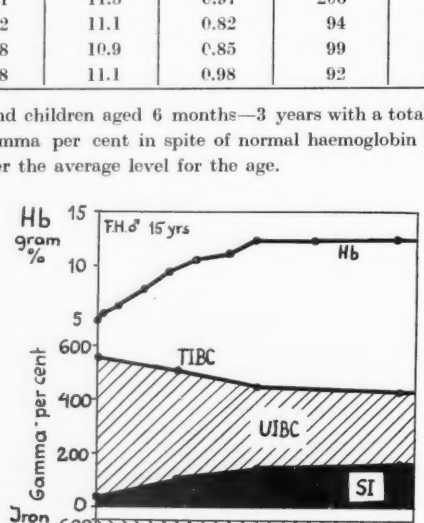

Fig. 10. *The changes in serum iron and total iron-binding capacity during oral iron therapy in a boy aged 15 years with an iron deficiency anaemia of unknown origin.*

values for the TIBC are still found long afterwards, probably indicating a continued readiness to mobilize and metabolize iron. This is in keeping with the very slow restoration of the iron stores found even with extensive oral iron treatment (HASKINS *et al.*, 1952).

5. Acute infectious diseases

The metabolism of iron is severely disturbed in infectious states. The mechanism of this and the changes in the serum iron have been studied by HEILMEYER (1937—41) and CARTWRIGHT, WINTROBE, and associates (1946—52)[1];

[1] For references see the review by CARTWRIGHT & WINTROBE (1952).

and on children by VAHLQUIST (1941), SCHÄFER (1940—49), and others. Investigations on the changes in the iron-binding capacity have been made by HOLMBERG & LAURELL (1945), LAURELL (1947), CARTWRIGHT & WINTROBE (1949), RATH & FINCH (1949), SMITH *et al.* (1952), and BRAUNSTEINER *et al.* (1952).

Acute infections are characterized by a low serum iron and a more or less diminished TIBC. Thus the TIBC is altered during infections just as in states with increased iron stores (fig. 1, p. 16) in spite of serum iron levels corresponding to those found in iron deficiency. Iron tolerance tests show that iron is eliminated from the blood plasma faster than normally. The normocytic anaemia which often accompanies infectious conditions seems to be due neither to changes in the TIBC nor to iron deficiency. The changes in the iron values and the anaemia of infection are best regarded as manifestations of a non-specific inflammatory reaction in the body. The same findings are also met with in experimentally produced aseptic inflammations (ROBSHEIT-ROBBINS & WHIPPLE, 1936; CARTWRIGHT & WINTROBE, 1949), in various toxic conditions, especially uraemia (LAURELL, 1947), and in most cases of malignant tumours (LAURELL, 1947).

The anaemia of infection seems to be caused mainly by impaired production of haemoglobin (WINTROBE *et al.*, 1947) combined with disturbed erythropoiesis (HEMMELER, 1946). Iron normally taken up in the bone marrow cannot be used there, and instead is diverted to reticulo-endothelial cells in the liver and spleen (SCHÄFER, 1942). Increased sternal marrow haemosiderin has also been observed (RATH & FINCH, 1948). In septic conditions there is also probably an increased rate of destruction of red cells (see for instance CARTWRIGHT & WINTROBE, 1952; HAGBERG, 1952) which gives a further surplus of iron. There is thus an increased deposition of iron in the usual storage organs (ROTH *et al.*, 1951), but this has little to do with normal storage. In fact, the reticulo-endothelial cells seem to have such a demand for iron that it disappears from the plasma at an increased rate, resulting in hypoferraemia. That this is the result of reticulo-endothelial activity is stressed by CARTWRIGHT *et al.* (1950), who demonstrated in dogs that hypoferraemia due to sterile turpentine abscesses could be abolished by a 'blockade' of the RES. Why this system removes iron from plasma during infection is not clear but the same authors (1951) suggest that the adrenal cortex may be concerned.

Whereas the principal changes in the serum iron and the iron-binding capacity during infection are well known and the cause of infectious hypoferraemia has been extensively studied, the factors influencing the extent and the rate of the changes in the TIBC have been far less elucidated. In this investigation the relation of these factors to the severity and duration of infec-

tion has been studied, and also the speed of the changes in comparison with the change in the serum iron.

Material. The TIBC was studied on samples collected from 82 children of all ages between 5 months and 14 years, at different stages of various acute infections. Children with incidental iron deficiency anaemia have been excluded as far as possible. All values refer to the morning fasting state. In most cases a single sample was taken, and was collected either when pyrexia was still present or just as it was beginning to abate. Some of the cases were examined repeatedly during the course of the disease and convalescence.

For statistical analysis the series was divided into two main groups. Children with pharyngitis, tonsillitis, sinusitis, uncomplicated lymphadenitis, and bronchitis, were referred to 'slight infections'; rheumatic fever, primary tuberculosis with pyrexia, pneumonia, septic lymphadenitis, suppurative otitis, and some other diseases of a similar degree of severity, were grouped as 'severe infections'.

Results. It appears from table XXIV that children with various infections of 2—3 days duration evidently have a TIBC which seems to be little or not at all changed. On the other hand the serum iron values were generally found to be low even on the first days of pyrexia.

Table XXV demonstrates that many of the cases with severe infectious diseases were found to have a lowered TIBC on the fourth to sixth day of the disease, whereas the corresponding values during slight infections were found

TABLE XXIV

Record no.	Sex Age yrs.	Diagnosis	Day of pyrexia	SI gamma per cent	TIBC gamma per cent
223/51	♂ 1	pharyngobronchitis	2	66	455
165/51	♀ 5	pharyngotonsillitis	2	70	430
941/52	♂ 3	pharyngitis	2	36	385
1185/50	♂ 2	pharyngobronchitis	2	36	375
115/51	♀ 6	bronchopneumonia	2	44	370
210/51	♀ 1/2	suppurative otitis media	2	64	365
1097/51	♀ 6	acute pyelitis	2	52	345
748/52	♀ 4	otitis media + pharyngobronchitis	3	34	340
840/51	♀ 8	tonsillitis	3	20	340
812/51	♀ 10	tonsillitis	2	42	325
46/51	♂ 3	pharyngitis	2	66	320
78/51	♂ 3 1/4	asthmathic bronchitis	3	64	305
865/51	♂ 12	bronchopneumonia	3	48	300
420/52	♀ 13 1/2	bronchopneumonia	2	37	295

The serum iron (SI) and the total iron-binding capacity (TIBC) in 14 non-anaemic infants and children with acute infections and pyrexia of 2—3 days duration.

TABLE XXV

Record no.	Sex Age yrs.	Diagnosis	Day of disease	ESR mm	TIBC gamma per cent
186/51	♂ 3	pharyngobronchitis	6	31	380
967/51	♂ 5 1/2	rhinopharyngitis	6	43	355
461/51	♀ 8	pharyngitis	6	25	355
84/52	♀ 4 3/4	tonsillitis + cervical lymphadenitis	6	34	350
831/51	♀ 6	acute mesenteric lymphadenitis	6	31	350
145/51	♂ 3 1/2	pneumonia	4	44	340
115/51	♀ 6	bronchopneumonia	6	9	340
1158/50	♂ 8 3/4	atypical pneumonia	5	35	335
978/52	♂ 8	pneumonia	4	49	320
717/51	♂ 7 1/4	pharyngobronchitis	5	19	320
840/51	♀ 8 1/4	tonsillitis	5	30	315
812/51	♀ 10 1/2	tonsillitis	6	46	315
978/51	♀ 7	atypical pneumonia	6	48	310
275/52	♂ 6 1/2	rheumatic fever	6	63	295
262/52	♀ 7 3/4	rheumatic fever	5	66	290
407/51	♂ 3 1/4	pharyngitis	4	41	285
88/51	♂ 4 1/4	pneumonia	4	48	270
420/52	♀ 13 1/2	bronchopneumonia	4	43	265
365/52	♀ 8 1/4	pneumonia	5	54	255
182/51	♀ 10 1/2	rheumatic fever	4	41	245
630/51	♀ 4 1/4	primary tuberculosis	4	55	235
105/51	♂ 13 3/4	acute pancarditis	4	65	230
846/52	♀ 4 1/2	tuberculous pleurisy	6	36	175

The total iron-binding capacity (TIBC) in 23 children aged 3—14 years with various infectious diseases. Sampling was done 4—6 days after the beginning of the pyrexia.

to be within normal limits but with a somewhat lower average level. Approximately the same results were found in cases with infectious diseases of 1—3 weeks duration, as can be seen in table XXVI; markedly lower values were not found in spite of the longer duration of infection.

The results of repeated estimations of TIBC during infection in individual cases (fig. 11 and table XXVII) confirm the findings in tables XXIV—XXVI. They also show that the return of the TIBC to normal seems to occur gradually within the week following recovery, that is to say usually somewhat more slowly than the serum iron (fig. 12).

Children suffering from infectious diseases with a protracted course seemed to have a TIBC which remained quite constant after an initial fall (fig. 13). This level varied from case to case and seemed to be determined by the severity of the disease rather than the actual diagnosis. Several children with lobar

TABLE XXVI

Record no.	Sex Age yrs.	Diagnosis	Week of disease	ESR mm	TIBC gamma per cent
1141/51	♀ 6	maxillar sinusitis + bronchitis	1 1/2	20	370
508/51	♀ 5 1/2	whooping cough + bronchopneumonia	1 1/2	41	350
967/51	♂ 5 1/2	pharyngobronchitis	1 1/2	43	345
489/51	♂ 3 1/2	pharyngolaryngitis	2	13	345
978/51	♀ 7	atypical pneumonia	1 1/2	48	340
163/51	♂ 8	atypical pneumonia	1	30	315
392/51	♂ 7	acute cervical lymphadenitis	1	45	310
1158/50	♂ 8 3/4	atypical pneumonia	2	40	310
538/52	♀ 10 3/4	rheumatic fever	1 1/2	81	300
312/52	♂ 4 3/4	acute mesenteric lymphadenitis	1	41	295
492/51	♂ 13	bronchopneumonia	1 1/2	45	290
825/51	♂ 7 3/4	bronchopneumonia	2	19	285
356/51	♂ 12 1/2	maxillar sinusitis	1 1/2	37	280
23/51	♀ 10 1/4	rheumatic fever	2	64	270
668/51	♀ 11 1/4	Löffler's syndrome	1 1/2	25	260
87/51	♂ 5 1/2	acute cervical lymphadenitis	1 1/2	62	245
920/52	♀ 8 1/4	rheumatic fever	3	60	235
417/52	♀ 7 1/4	primary tuberculosis	2	66	235
1169/50	♂ 6 3/4	exsudative pleurisy	>3	35	225
615/51	♀ 7 1/2	atypical pneumonia	2	45	200

The total iron-binding capacity (TIBC) in 20 children aged 3—14 years with various infectious diseases of 1—3 weeks duration.

or bronchopneumonia but in good general condition had a TIBC within normal limits, whereas in those severely ill it was often found to be low. The lowest values obtained (TIBC = 175 gamma per cent) were in a 2-year-old boy critically ill with an atypical pneumonia, and a 4-year-old girl with a severe tuberculous pleurisy and toxic signs.

Tables XXVIII and XXIX record the statistical analysis of the effect of the severity and duration of infectious diseases upon the TIBC. It is obvious (table XXVIII) that severe infections reduce the TIBC far below the level of healthy children in the same age group. The difference (75 ± 9.5 gamma per cent) is statistically significant. The mean value in 'slight infections' is also less than that in healthy children. The difference (23 ± 8.1 gamma per cent) is statistically probable but might be significant in a larger series.

Table XXIX demonstrates that the fall in TIBC due to severe infections was significantly greater than that in slight infectious diseases. The difference was apparent during the first four days of the disease, but was not statistically significant until the infection had lasted five days or more. Compared with

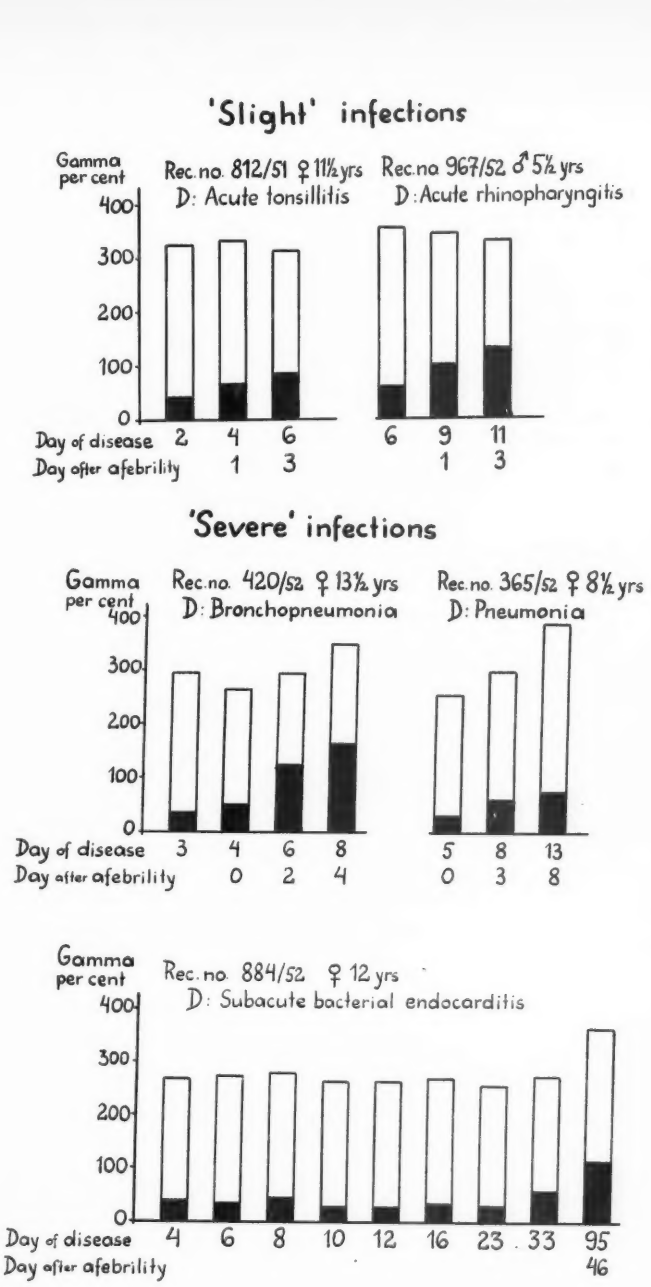

Fig. 11. *Representative examples of infected children in whom repeated determinations of the serum iron and the total iron-binding capacity were made.*

TABLE XXVII

Sex Age Rec. no.	Day of disease	Day after afebrility	Day of ACTH treatment	ESR mm	SI gamma per cent	TIBC gamma per cent
	6			63	67	295
♂	9	0	3		67	310
	12	3	6	59	62	305
6 1/2 yrs.	15	6	9		61	385
	20	11	14	32	130	415
275/52	41	32	—	16	100	420
	56	47	—	11	83	405
	19	2	4	60	82	235
♀	22	5	7		52	285
8 yrs.	25	8	10	30	90	295
38/52	30	13	15	17	90	350
	70	53	—	8	109	345
♀	5	0	1	66	84	290
7 3/4 yrs.	10	5	6	32	116	355
262/52						
♀	4			41	62	245
10 1/2 yrs.	13	6	8	55	72	310
182/51						

The changes in serum iron (SI) and total iron-binding capacity (TIBC) in patients with rheumatic fever in remission during ACTH-treatment.

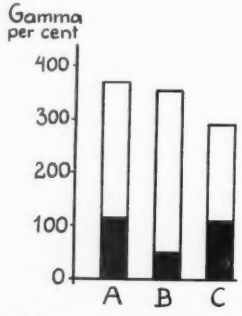

Fig. 12. *The fall in serum iron takes place during the first day of infection and is rapidly restored on recovery. The changes in TIBC occur more slowly.*

A. 73 healthy children aged 1—14 years.

B. 14 children with acute infections and pyrexia for three days or less (table XXIV).

C. 14 children on the first day of afebrility after severe infectious diseases of five days duration or more.

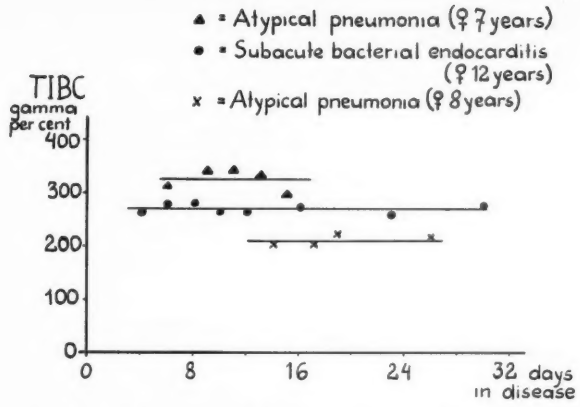

Fig. 13. *Three girls followed throughout the course of infectious diseases. After an initial fall the TIBC remains on a fairly constant level until recovery.*

TABLE XXVIII

Children 1—14 yrs.	No. of cases	TIBC gamma per cent M ± ε (M)	Decrease in comparison with healthy subjects
Healthy	73	368 ± 5.3	—
Slightly infected.....	33	345 ± 6.1	23 ± 8.1
Severely infected	49	293 ± 7.9	75 ± 9.5

The decrease in the total iron-binding capacity (TIBC) during slight and severe infectious diseases in comparison with the values in healthy children.

TABLE XXIX

Day of disease	Age yrs.	Slight infections		Severe infections		P
		No. of cases	TIBC gamma per cent M ± ε (M)	No. of cases	TIBC gamma per cent M ± ε (M)	
1—4	1— 5	11	349	8	332	>0.05
	5—14	5	355	9	300	>0.05
	1—14	16	351 ± 9.7	17	315 ± 15.1	>0.05
5—ω	1— 5	8	351	11	282	0.01—0.001
	5—14	9	331	21	279	0.01—0.001
	1—14	17	340 ± 7.6	32	280 ± 8.4	<0.001
	All children	33	345 ± 6.1	49	293 ± 7.9	<0.001

The changes in the total iron-binding capacity (TIBC) during slight and severe infectious diseases of varying duration.

TABLE XXX

ESR mm	Age group (yrs.)	No. of cases	TIBC gamma per cent M ± ε (M)
<30	<5	12	349
	5 and over	8	346
	All ages	20	348 ± 12.7
30—50	<5	17	319
	5 and over	20	306
	All ages	37	312 ± 8.7
>50	<5	12	300
	5 and over	10	275
	All ages	22	289 ± 10.2

The relation between the decrease in the total iron-binding capacity (TIBC) and the increase in the erythrocyte sedimentation rate (ESR) in children with infectious diseases.

healthy children of the same age there was, however, a statistically significant fall in the TIBC during the first four days of severe infectious disease.

The results recorded in table XXX confirm once more that the severity of the infection is of importance. Cases with an erythrocyte sedimentation rate (ESR) of more than 50 mm were found to have a lower TIBC than those in whom it was less than 30 mm. The difference (59 ± 16.3) is statistically significant. There seems, however, to be no consistent correlation between a high sedimentation rate and a low TIBC. Some cases had an only moderately increased sedimentation rate but a markedly lowered TIBC (see tables XXV and XXVI).

No consistent connection between the changes in the TIBC and the degree of pyrexia was found. Some of the lowest TIBC values were observed in cases with moderate pyrexia of relatively short duration, whereas a boy aged ten with a reticulum cell sarcoma (rec. no. 47/52) and continuous fever of about 39—40° C for over one month had a low normal TIBC (285 gamma per cent).

Discussion and conclusions. It has been confirmed that acute infectious diseases diminish the TIBC. The changes in children do not seem to be more pronounced than in adults, to judge from the results of other observers. In the first place the extent of the changes seems to depend upon the severity of the disease. Slight infections such as common pharyngitis usually bring about insignificant changes in the TIBC, whereas prostrating infections are most often associated with considerably lowered values. This is not contradicted by the wide range of variation in the TIBC found in cases with the

same diagnosis, as the lowest values generally seem to be found in patients most severely ill. Similarly, most cases with pyrexia and a markedly increased erythrocyte sedimentation rate have a lowered TIBC, and here again there is no definite correlation.

In spite of a predominance of slight infections of short duration and severe infections with a longer course, the results give an idea of the changes in the TIBC due to the duration of the infection. It thus seems to fall steadily from the onset, reaching a level of equilibrium within the first week. The rate of this initial fall and its total extent apparently depend in the first place on the severity of the infection, but individual factors may also be of importance. Once the level of equilibrium is attained the TIBC seems to remain fairly constant until the infection abates. There is then a gradual return to normal, probably lasting several days.

It has not been possible to determine whether the post-infectious rise in the TIBC temporarily exceeds the pre-infectious level. Such an alteration seems reasonable in the light of the reversal of the plasma iron transport during recovery. From the cases investigated it was impossible to evaluate fully the importance of the pre-infectious level in determining the degree of the subsequent fall in TIBC. Very low values have, however, been observed in infected cases in whom a definite iron deficiency existed, which suggests that the infection was predominant. A similar dominance of changes due to infection also applies to the serum iron (VAHLQUIST, 1941).

The results support the assertion of CARTWRIGHT & WINTROBE (1949) that the drop in transferrin during infection does not cause the hypoferraemia. This is finally proved by the fact that the fluctuation in serum iron seems to occur more rapidly than that in the TIBC. Thus definitely low serum iron values were found during the second day of illness, when the TIBC was in all cases still within normal limits, and the average was only slightly lowered. On the other hand in many cases the serum iron apparently returns to normal by the first day of afebrility, whereas the TIBC is still low. Thus the changes in the serum iron and the TIBC during infections seem to be independent.

The cause of the hypoferraemia during infectious states seems to be the increased demand of iron by the RES, the reason for which is hard to explain. The fall in the TIBC seems to be better interpreted as a regulative mechanism during a reversed iron metabolism than as a result of disturbed protein synthesis. It is tempting to assume that the fall is an expression of the increased deposition of iron in the storage organs during infection, the extent depending on the severity of the disturbance as the amount of iron available for deposition is probably determined by the degree of erythropoietic depression and the extent of break-down of red cells.

SUMMARY

Changes in the iron metabolism are reflected in the plasma and can be estimated from fluctuations in the iron-transporting plasma protein, transferrin, and its amount of attached iron, the serum iron.

This study refers to investigations on the serum iron and the total iron-binding capacity of the transferrin in about 300 infants and children. Special emphasis has been laid on the physiological changes occurring during infancy, when the amount of storage iron changes more than at any other time in later life. The iron stores, satisfactory at birth, increase during the first two months, but thereafter fall successively, remaining small during the next few years.

Methods

In estimating the total iron-binding capacity, the iron-saturated fraction (the serum iron) and the unsaturated fraction (the unsaturated iron-binding capacity) were determined independently and added together.

For the determination of the serum iron a modification of the method of VAHLQUIST (1941) was used and found sufficiently accurate. Only 0.3 ml of serum was needed. Instead of filtering, the sample was centrifuged after precipitation of the proteins.

The unsaturated iron-binding capacity was determined according to the method of RATH & FINCH (1949), which is based on the fact that the salmon-red iron-transferrin colour deepens on fractionated addition of iron until full saturation is attained. This method was found to have definite limitations. It was unreliable in lipaemic sera or sera with discoloration or severe disturbance of the plasma protein pattern. If the concentration of bilirubin exceeded 3—4 mg per cent the estimation could not be satisfactorily performed, whereas slight haemolysis did not interfere with the result. In spite of its disadvantages the method was, however, found to be accurate enough for the special purposes of this investigation, a fact proved by iron tolerance tests.

A simple 'one stage measurement' of the total increase in extinction after supersaturation with iron was found to give too unreliable values, probably owing to the presence of variable amounts of substances interfering with the development of the iron-transferrin colour. Possibly due to the same mechanism an average introductory iron enrichment of 15 gamma per 100 ml was found to be necessary to obtain any increase in extinction at all.

Clinical investigations

1. The average serum iron level in 54 healthy adults (26 men and 28 women) was 130 ± 5.2 gamma per cent, and the corresponding total iron-binding capacity 330 ± 4.9 gamma per cent. These figures served as basic values for judging the results obtained in infants and children.

2. The serum iron of the *umbilical cord* at parturition was as high as 173 ± 6.9 gamma per cent, whereas *the total iron-binding capacity was 259 ± 10.5, i.e. significantly less than in healthy adults. The changes parallel a stage of active storage of iron*, and are in striking contrast to the pattern in the serum of the mothers, in whom there is mobilization of iron from the depots.

3. *Infants $^1/_2$—2 months* of age are in a state of physiologically suppressed erythropoietic activity, which implies a diminished need and hence *increased storage of iron*. They showed *a still lower total iron-binding capacity*, 212 ± 6.6 gamma per cent, and an average serum iron of 142 ± 7.1 gamma per cent, i.e. significantly less than at birth but still high. It is notable that *the relative saturation with iron was the same as in cord blood.*

4. *Infants aged 2—6 months*, the period of return to full erythropoietic activity during which *increasing amounts of iron are mobilized from the depots*, had a falling serum iron (113 ± 5.6 gamma per cent at 2—4 months, and 78 ± 6.1 at 4—6 months) and *a rising total iron-binding capacity* (308 ± 11.3 gamma per cent at 2—4 months, and 360 ± 12.5 at 4—6 months).

5. The serum iron values in *infants and children of $^1/_2$—3 years* remained low, with an average of about 95 gamma per cent, and *the total iron-binding capacity was markedly elevated*, with an average of about 390 gamma per cent. *It is at these ages that the iron stores are minimal*, and the children are particularly vulnerable to even small additional strains on the iron balance. The values obtained put in relation to the haemoglobin level also suggested that some cases with slight iron deficiency anaemia were included in a 'normal' series in spite of haemoglobin values within accepted limits.

6. The values in *older children successively approached adult levels*, which were attained more rapidly by the serum iron than by the total iron-binding capacity.

7. A series of 16 children with *iron deficiency anaemia* had an average serum iron of 50 ± 6.5 gamma per cent, and a total iron-binding capacity of 496 ± 7.1 gamma per cent.

8. *The general conclusion was that in infants and children the level of the total iron-binding capacity and its saturation with iron closely reflects the probable direction of the plasma iron transport, i.e. the storage and mobilization of iron, and thus usually also the size of the iron depots. The findings clearly support* LAURELL's *hypothesis concerning the regulation of the plasma transport of iron.*

9. The metabolism of iron is disturbed during *infection*. The disturbance is revealed by a simultaneous fall in the serum iron and total iron-binding capacity. This pattern of change deviates from the physiological outlined above.

From studies in 82 children suffering from various acute infections it was found that *the extent and rate of the fall in total iron-binding capacity was mainly related to the severity of the infection*. It developed successively during the first week, and reached a level of equilibrium which remained constant in infections with a protracted course. The return to normal seemed to occur gradually within the week following recovery.

It was also found that *the changes in serum iron generally occurred more rapidly than those in the total iron-binding capacity*. This confirmed earlier assumptions that the lowering of the total iron-binding capacity does not cause the hypoferraemia of infection.

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Résumé

La capacité du serum de lier le fer chez les nourrissons et les enfants

Les changements dans le métabolisme du fer se reflètent dans le plasma et peuvent être déterminés à partir des oscillations dans le plasma protéineux, la transferrine (qui transporte le fer), et dans la masse de fer qui y est attaché, le fer sérique.

Cette étude concerne les recherches, conduites chez environ 300 nourrissons et enfants, sur le fer sérique et la capacité totale qu'a la transferrine de lier le fer. On a mis spécialement l'accent sur les changements physiologiques qui se produisent au cours de l'enfance lorsque la masse disponible de fer change plus qu'à n'importe quel autre moment de la vie. Les réserves de fer, suffisantes à la naissance, augmentent au cours des deux premiers mois, mais, après, diminuent peu à peu et restent faibles au cours des premières années suivantes.

Méthodes

En estimant la capacité totale de lier le fer, la fraction saturée de fer (le fer sérique) et la fraction non saturée (la capacité de lier le fer non saturée) furent déterminées indépendamment, et additionnées. Pour déterminer le fer sérique, on s'est servi d'une modification de la méthode Vahlquist (1941) qui s'est révélée suffisamment exacte. Il ne fallut que 0.3 ml de serum. Au lieu du filtrage, une centrifugation fut faite après la précipitation des protéines.

La capacité de lier le fer non saturée fut déterminée d'après la méthode Rath et Finch (1949) qui se base sur le fait que la couleur rouge saumon de la transferrine du fer se fonce après une addition graduée de fer jusqu'à ce que pleine saturation soit atteinte. On a constaté, que cette méthode a des limites définies. Elle était inexacte dans les serums lipémiques et les serums avec décoloration ou perturbation considérable du type du plasma protéineux. Si la concentration de bilirubine dépassait 3 à 4 mg pour cent, l'estimation ne pourrait être évaluée de manière satisfaisante, tandis que une hémolyse légère ne modifie pas le résultat. Cependant, malgré ses désavantages, la méthode se révéla suffisamment exacte pour le but que nous poursuivions: ce qui a été prouvé par des recherches de la tolérance du fer.

La "simple mesure d'une phase" de l'augmentation totale en extinction après supersaturation de fer se trouva donner des résultats trop douteux,

probablement à cause de la présence de masses différentes de substances qui interfèrent avec le développement de la couleur transferrine du fer.

Probablement dû au même mécanisme, un enrichissement préliminaire de fer de 15 γ par 100 ml en moyenne se trouva nécéssaire pour obtenir n'importe quelle augmentation en extinction.

Recherches cliniques

1. Le niveau moyen du fer sérique chez 54 adultes (26 hommes et 28 femmes) en bonne santé fut 130 ± 5.2 γ% et la capacité correspondante totale de lier le fer 330 ± 4.9 γ%. Ces chiffres ont servi de valeurs de base pour juger les résultats obtenus chez enfants et nourrissons.

2. Le fer sérique du *cordon ombilical* s'élevait à 173 ± 6.9 γ% à la naissance, tandis que *la capacité totale de lier le fer fut* 259 ± 10.5 γ%, *c-à-d considérablement moins que chez des adultes en bonne santé. Les changements sont parallèles à une phase d'emmagasinement actif de fer* et contrastent violemment avec le type du serum maternel où il y a une libération de fer à partir des dépôts.

3. *Les nourrissons âgés de deux semaines à deux mois* sont dans un état d'activité érythropoiétique physiologiquement représsive, ce qui implique un besoin diminué, et, désormais *une augmentation du stock de fer*. Ils montraient *une capacité totale de lier le fer encore plus basse*, $212 \pm 6{,}6$ γ%, et un fer sérique de 142 ± 7.1 γ% en moyenne, c-à-d une moyenne considérablement moindre qu'a la naissance mais pourtant élevée. Il est à remarquer que *la saturation de fer relative était la même que dans le sang du cordon.*

4. *Les nourrissons âgés de 2 à 6 mois*, période de retour à une pleine activité érythropoiétique pendant laquelle des *masses de fer croissantes sont libérées des dépôts*, avaient un fer sérique décroissant (113 ± 5.6 γ% de 2 à 4 mois, et 78 ± 6.1 γ% de 4 à 6 mois) et *une capacité totale de lier le fer progressive* (308 ± 11.3 γ% de 2 à 4 mois, et 360 ± 12.5 γ% de 4 à 6 mois).

5. Les valeurs du fer sérique chez *les nourrissons et chez les enfants âgés de six mois à trois ans*, restaient basses, environ 95 γ% en moyenne, et *leur capacité totale de lier le fer était remarquablement élevée*, environ 390 en moyenne. *C'est à cet âge que les réserves de fer sont infimes*, et que les enfants sont spécialement vulnérables même aux moindres surcharges de la balance du fer. Les valeurs obtenues mises en relation avec le niveau d'hémoglobine montraient aussi que quelques cas d'anémie ferriprive légère étaient inclus dans une série "normale" malgré des valeurs d'hémoglobine qui restent dans des limites acceptées.

6. Les valeurs chez *des enfants plus âgés s'approchaient peu à peu des niveaux d'adultes* qui étaient plus vite atteints par le fer sérique que par la capacité totale de lier le fer.

7. Une série de 16 enfants *d'anémie ferriprive* avait un fer sérique de 50 ± 6.5 γ% en moyenne, et une capacité totale de lier le fer de 496 ± 7.1 γ%.

8. La conclusion générale fut que chez les nourrissons et chez les enfants, le niveau de la capacité totale de lier le fer, et sa saturation de fer reflètent de près la direction probable du transport du fer du plasma, c-à-d emmagasinement et libération de fer, ainsi qu'ordinairement aussi le volume des dépôts de fer. Les découvertes supportent avec précision l'hypothèse Laurell en ce qui concerne la régularisation du transport de fer dans le plasma.

9. Le métabolisme du fer est troublé au cours d'une *infection*. La perturbation se révèle par une réduction simultanée dans le fer sérique et par une réduction de la capacité totale de lier le fer. Ce type de changement diffère du comportement physiologique esquissé ci-dessus.

Par des études conduites sur 82 enfants souffrant de diverses infections aiguës, il est apparu que *l'extension et la rapidité de la réduction de la capacité totale de lier le fer eurent surtout rapport au degré de gravité de l'infection*. La réduction s'accentua peu à peu durant la première semaine et atteignit un niveau d'équilibre qui resta constant lors d'infections d'une durée assez longue. Le retour à l'état normal semble s'être fait graduellement dans la semaine qui a suivi la guérison.

On a trouvé aussi que *les changements dans le fer sérique en général ont lieu plus rapidement que ceux de la capacité totale de lier le fer*. Cela confirma les hypothèses précédentes que la réduction de la capacité totale de lier le fer ne cause pas l'hypoferrémie d'infection.

Zusammenfassung

Das Eisenbindungsvermögen des Serums bei Säuglingen und Kindern

Die Änderungen des Eisenumsatzes spiegeln sich im Plasma ab und können von den Schwankungen des eisentragenden Plasmaproteins, das Transferrin, und seinem Betrag an gebundenem Eisen bestimmt werden.

Diese Arbeit bezieht sich auf Versuche am Serumeisen und am totalen Eisenbindungsvermögen des Transferrins bei ungefähr 300 Säuglingen und Kindern. Besonderer Nachdruck wurde auf die physiologischen Schwankungen während der Kindheit gelegt, wenn die Menge des zur Verfügung stehenden Depoteisens mehr als je im späteren Leben wechselt. Die Eisenvorräte, welche bei der Geburt befriedigend sind, nehmen während der zwei ersten Monate zu, werden aber allmählich erschöpft und bleiben in den nächsten darauffolgenden Jahren gering.

Methoden

Zur Bestimmung des totalen Eisenbindungsvermögens wurden die eisengesättigte Fraktion (das Serumeisen) und die ungesättigte (das ungesättigte Eisenbindungsvermögen) unabhängig voneinander bestimmt und nachher zusammen addiert.

Zur Bestimmung des Serumeisens wurde eine Abwandlung der Methode von VAHLQUIST (1941) angewandt und für genügend exakt befunden. Es wurden nur 0,3 ml Serum hinzugefügt. Anstatt zu filtrieren, wurde nach der Fällung der Proteine zentrifugiert.

Das ungesättigte Eisenbindungsvermögen wurde nach der Methode von RATH & FINCH (1949) bestimmt, welche sich auf die Tatsache gründet, dass die rote Lachsfarbe des Eisentransferrins sich bei schrittweisem Zusatz von Eisen vertieft bis die Sättigung erreicht wird. Dagegen war sie bei Fettsera oder bei verfärbten Sera oder bei Sera mit schweren Störungen des Plasmaeiweissgerüstes unzuverlässig. Wenn die Konzentration des Bilirubins 3—4 mg Prozent überstieg, konnte die Bestimmung nicht genau ausgeführt werden, während eine schwache Hämolyse das Resultat nicht störte. Trotz dieser Nachteile wurde die Methode für die speziellen Absichten dieser Versuche für genügend genau gefunden, ein Faktum, das durch Eisenverträglichkeitstests bewiesen wurde.

Eine einfache "einmalige Messung" der totalen Zunahme der Extinktion nach Übersättigung mit Eisen wurde für zu unzuverlässig befunden. Wahrscheinlich hängt das von veränderlichen Mengen von Substanzen ab, welche die Entwicklung der Eisentransferrinfarbe hemmen. Möglicherweise abhängig vom selben Mechanismus ist die Tatsache, dass eine einleitende Durchschnittseisenbereicherung von 15 γ per 100 ml als notwendig gefunden wurde, um Überhaupt eine Zunahme der Extinktion zu erhalten.

Klinische Untersuchungen

1. Der Durchschnitt des Serumeisenspiegels bei 54 gesunden Erwachsenen (26 Männer und 28 Frauen) war $130 \pm 5{,}2\ \gamma\%$ und das entsprechende totale Eisenbindungsvermögen $330 \pm 4{,}9\ \gamma\%$. Diese Bilder dienen als grundlegende Werte für die Beurteilung der bei Säuglingen und Kindern erhaltenen Resultate.

2. Der Serumeisengehalt der *Nabelschnur* war bei der Geburt $173 \pm 6{,}9\ \gamma\%$, während *das totale Eisenbindungsvermögen* $259 \pm 10{,}5\ \gamma\%$ *war, d.h. bedeutend niedrigere Werte als die bei Erwachsenen aufwies. Die Veränderungen gehen parallel mit einer aktiven Eisenspeicherungsstelle* und stehen in auffallendem Kontrast zu dem Bild in dem Serum der Mütter, mit deren erhöhte Eisenmobilisation aus den Depots.

3. *Säuglinge im Alter von $^1/_2$—2 Monaten* befinden sich in einem Stadium von physiologisch gesenkter erythropoietischer Aktivität, was ein geringeres Bedürfnis an Eisen und damit eine *erhöhte Eisenspeicherung* bedeutet. Sie zeigten *ferner eine Senkung des totalen Eisenbindungsvermögens*, 212 ± 6,6 γ%, und einen Serumeisendurchschnittswert von 142 ± 7,1 γ%, d.h. bedeutend niedriger als bei der Geburt, aber noch hoch. Es ist auffallend, dass *die entsprechende Sättigung mit Eisen dieselbe wie in der Nabelschnur war.*

4. *Kinder im Alter von 2—6 Monaten*, d.h. wenn die volle erythropoietische Aktivität langsam wiederkommt und *eine wachsende Eisenmenge von den Depots mobilisiert wird*, hatten einen Abfall des Serumeisens (113 ± 5,6 γ% im 2.—4. Monat, und 78 ± 6,1 γ% im 4.—6. Monat) und ein *zunehmendes totales Eisenbindungsvermögen* (308 ± 11,3 γ% im 2.—4. Monat und 360 ± 12,5 γ% im 4.—6. Monat).

5. Die Serumeisenwerte bei *Säuglingen und Kindern zwischen $^1/_2$—3 Jahren* blieben niedrig mit einem Durchschnittswert von ungefähr 95 γ%. *Ihr totales Eisenbindungsvermögen war auffallend hoch*, der Durchschnittswert war ungefähr 390. *Es ist in diesen Altersgruppen bei denen die Eisenspeicherung minimal ist* und die Kinder besonders empfindlich auch für kleine Schwankungen des Eisengleichgewichts sind. Setzt man die erhaltenen Werte in Beziehung zu dem Hemoglobinspiegel, so deuten sie an, dass einige Fälle mit einer leichten Eisenmangelanämie in die "normalen" Serien aufgenommen wurden, trotzdem die Hämoglobinwerte innerhalb annehmbarer Grenzen liegen.

6. *Die Werte der älteren Kinder näherten sich allmählich den Werten der Erwachsenen* und wurden vom Serumeisen schneller als vom totalen Eisenbindungsvermögen erreicht.

7. Eine Serie von 16 Kindern mit *Eisenmangelanämie* hatte einen Serumeisendurchschnittswert von 50 ± 6,5 γ% und ein totales Eisenbindungsvermögen von 496 ± 7,1 γ%.

8. Es wurde der allgemeine Schluss gezogen, dass bei Säuglingen und Kindern die Höhe des totalen Eisenbindungsvermögens und seine Sättigung mit Eisen die wahrscheinliche Richtung des Plasmaeisentransports nahe wiederspiegelt, d.h. die Aufspeicherung und Mobilisation von Eisen und damit also gewöhnlich den Umfang der Eisendepots.

9. Der Eisenstoffwechsel ist während *Infektionen* gestört. Die Störung äussert sich, unter anderen Dingen, durch einen Abfall des Serumeisens und zur gleichen Zeit eine Senkung des totalen Eisenbindungsvermögens. Dieses Bild von Veränderungen weicht von dem weiter oben beschriebenen physiologischen Bild ab.

Bei an 82 Kindern gemachten Untersuchungen, die an verschiedenen akuten Infektionen litten, wurde gefunden, dass *der Umfang und das Mass des Abfalles des totalen Eisenbindungsvermögens hauptsächlich zu der Schwere*

der Infektion in Beziehung standen. Er entfaltete sich allmählich während der ersten Woche und erreichte einen Gleichgewichtsspiegel, welcher sich bei Infektionen mit protrahiertem Kurs konstant hielt. Die Rückkehr zu normalen Werten schien im Bereich der ersten Woche nach der Wiederherstellung schrittweise einzufallen.

Es wurde auch gefunden, dass die *Veränderungen des Serumeisens im allgemeinen schneller vor sich gingen als diejenigen des totalen Eisenbindungsvermögens.* Das bestätigt frühere Behauptungen, dass die Senkung des totalen Eisenbindungsvermögens nicht die Ursache der Hypoferremia der Infektion sein kann.

Resumen

La capacidad de fijación de hierro del suero en lactantes y niños

Los cambios del metabolismo del hierro se reflejan en el plasma y pueden valorarse de las fluctuaciones de la proteina transportadora del hierro, la transferrina, y de la cantidad de hierro a ella ligada, a saber el hierro del suero.

Este estudio se refiere a investigaciones sobre el hierro del suero y la capacidad de fijación total de hierro de la transferrina en 300 lactantes y niños aproximadamente. Se ha puesto un interés especial en los cambios fisiológicos durante la infancia, tiempo en el que la cantidad de reservas de hierro disponibles varia más que en edades más avanzadas. Las reservas de hierro, satisfactorias al nacer, aumentan durante los dos primeros meses y van disminuyendo después sucesivamente, durante los primeros próximos años.

Métodos

Para determinar la capacidad total de fijación de hierro, se analizaron la fracción de hierro saturada (el hierro suérico) y la insaturada (la capacidad de fijación de hierro insaturada) cada una por si sola y sumándose después.

Para analizar el hierro suérico se usó una modificación del método de VAHLQUIST (1941) encontrándose suficientemente exacta. Solo se añadieron 0,3 ml de suero y en lugar de filtrar se centrifugó después de la precipitación de las proteinas.

La capacidad de fijación de hierro insaturada se determinó según el método de RATH & FINCH (1949), que se basa en el hecho de que el color rojo asalmonado de la transferrina férrica se intensifica con la adición graduada de hierro hasta que se ha alcanzado la saturación total. Se encontró que este método tiene ciertas limitaciones. De este modo resultó ser inexacto en sueros lipémicos, sueros decolorados o en graves trastornos del armazón de la proteina plasmática. Si la concentración de la bilirubina excedía unos 3—4 mg por

ciento, el análisis no podía llevarse a cabo satisfactoriamente, mientras que una hemolisis débil no estorbaba el resultado. A pesar de estas desventajas el método se encontró lo suficientemente exacto para los propósitos especiales de estas investigaciones, hecho probado a través de tests de tolerancia férrica.

Se encontró que la simple medida de una fase del aumento total de la extinción después de la supersaturación con hierro daba resultados inexactos. Es probable que esto se deba a la presencia de cantidades variables de substancias que inhiben la evolución del color de la transferrina férrica. Posiblemente debido al mismo mecanismo se encontró que es necesario un enriquecimiento de hierro medio de 15 γ por 100 ml para llegar a obtener un aumento de la extinción.

Investigaciones clínicas

1. El promedio de hierro suérico en 54 adultos sanos (26 hombres y 28 mujeres) fué de $130 \pm 5{,}2$ γ % y la capacidad total de hierro respectiva $330 \pm 4{,}9$ γ %. Estas figuras sirven de valores básicos para juzgar los resultados obtenidos en lactantes y niños.

2. El hierro suérico del *cordón umbilical* en el parto fué de $173 \pm 6{,}9$ γ %, mientras que la *capacidad total de fijación de hierro fué de* $259 \pm 10{,}5$ γ %, *es decir mostró valores más bajos que en adultos sanos.* Los cambios van paralelos a un almacenamiento activo de hierro y están en contraste violento con el cuadro del suero de las madres donde hay una liberación de hierro de los depósitos.

3. *Lactantes de* $^1/_2$*—2* meses de edad se encuentran en un estado de actividad eritropoyética fisiológica baja, lo que significa una necesidad disminuida de hierro y de este modo un *aumento de los depósitos.* Muestran *además una disminución de la capacidad total de fijación de hierro,* $212 \pm 6{,}6$ γ % y un promedio de hierro suérico de $142 \pm 7{,}1$ γ %, es decir marcadamente más bajo que al nacer, pero sin embargo alto. Es notable que la saturación relativa de hierro fuera la misma que la del cordón umbilical.

4. *Los lactantes de 2—6 meses,* es decir cuando la actividad eritropoyética total vuelve sucesivamente y *se moviliza un aumento creciente de hierro de los depósitos,* mostraron una baja del hierro suérico ($113 \pm 5{,}6$ γ % de los 2—4 meses y $78 \pm 6{,}1$ γ % de los 4—6 meses) y un *aumento de la capacidad total de fijación de hierro* ($308 \pm 11{,}3$ γ % de los 2—4 meses y $360 \pm 12{,}5$ γ % de los 4—6 meses).

5. Los valores de hierro suérico de *lactantes y niños entre* $^1/_2$*—3 años* se mantuvieron a un nivel más bajo con un promedio de 95 γ % aproximadamente. *Su capacidad total de fijación de hierro fué extraordinariamente elevada* y el promedio de unos 390. Justamente en estas edades los depósitos de hierro son minimales y los niños particularmente sensibles incluso a pequeñas

alteraciones adicionales del balance del hierro. Si se ponen los valores obtenidos en relación con la hemoglobina, estos indican también que algunos casos con una anemia hipoférrica débil fueron incluidos en series "normales" a pesar de mostrar valores hemoglobínicos dentro de límites aceptables.

6. *Los valores en los niños de más edad se aproximan sucesivamente a los de los adultos* que fueron alcanzados más rápidamente por el hierro suérico que por la capacidad total de fijación de hierro.

7. Una serie de 16 niños con *anemia hipoférrica* mostraron un valor promedio de hierro suérico de $50 \pm 6{,}5\ \gamma\%$ y una capacidad total de fijación de hierro de $496 \pm 7{,}1\ \gamma\%$.

8. Se sacó la conclusión de que el nivel de la capacidad total de fijación de hierro y su saturación de hierro en lactantes y niños reflejan la dirección probable del transporte de hierro plasmático, es decir el almacenamiento y la movilización de hierro así como la magnitud de los depósitos de hierro. Los resultados apoyan claramente la hipótesis de Laurell referente a la regulación del transporte del hierro plasmático.

9. El metabolismo del hierro está alterado durante las *infecciones* y entre otras cosas se revela por una baja en el hierro suérico y al mismo tiempo por una disminución de la capacidad total de fijación de hierro. Este cuadro de variaciones difiere del cuadro fisiológico más arriba descrito.

De estudios de 82 niños que sufrían distintas infecciones agudas se sacó la conclusión de que la *extensión y la velocidad de la baja de la capacidad total de fijación de hierro estuvo principalmente relacionada con la gravedad de la infección.* Se desarrolló sucesivamente durante la primera semana y alcanzó un equilibrio que se mantuvo constante en infecciones con un curso protrahido. La vuelta a valores normales pareció ocurrir gradualmente durante la semana siguiente al restablecimiento.

Se encontró también que los *cambios del hierro suérico ocurren generalmente con más rapidez que aquellos de la capacidad total de fijación de hierro.* Esto confirma las suposiciones anteriores de que la baja de la capacidad total de fijación de hierro no es la que causa la hipoferremia de infección.

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THE LONG-TERM PROGNOSIS FOR PREMATURELY BORN CHILDREN

A Follow-Up Study of 999 Premature Boys Born in Wedlock and of 1002 Controls

BY

INGVAR ALM

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FROM THE PAEDIATRIC CLINIC OF KAROLINSKA SJUKHUSET, STOCKHOLM, SWEDEN
(*Head:* PROFESSOR ARVID WALLGREN, M.D.)
and
THE STATE INSTITUTE OF HUMAN GENETICS AND RACE BIOLOGY, UPPSALA, SWEDEN
(*Head:* PROFESSOR GUNNAR DAHLBERG, M.D., LL.D.)

THE LONG-TERM PROGNOSIS FOR PREMATURELY BORN CHILDREN

A Follow-Up Study of 999 Premature Boys Born in Wedlock and of 1002 Controls

By

INGVAR ALM

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To the Memory of

J. HENNING MAGNUSSON, M.D.

Late Physician-in-Chief, The Sachs' Hospital
for Children, Stockholm, Sweden

PREFACE

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Stockholm, March 1953.

INGVAR ALM

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CHAPTER I

Definition

In the present paper, a premature infant denotes an infant who weighs 2500 grams or less at birth. This definition in terms of weight has been used intermittently since the eighteen-sixties. It was motivated at an early stage on the grounds that newborn infants with a lower birth weight presented special problems, particularly of a paediatric nature, which distinguished them from infants with a higher birth weight. It was, however, only after Ylppö's three publications in 1919 that this weight became more generally accepted as a clinical standard of prematurity, at any rate by paediatricians. For practical reasons, the 5 gram groups above and below 2500 grams, respectively, were soon included. In order to establish a uniform classification, the American Academy of Paediatrics passed the following resolution in 1935:

"A premature infant is one who weighs 2500 grams or less at birth (not at admission) regardless of the period of gestation."

This borderline figure was also recommended by the Committee on Hygiene of the League of Nations in 1937 and by the World Health Organization in 1950. The latter organization proposed the term *immaturity*, stating that: "An immature infant is a liveborn infant with a birth weight of 5 $^1/_2$ lb. (2500 Gm.) or less or specified as immature (a period of gestation of less than 37 weeks)." The term immaturity had, however, already been suggested by Ylppö[1] for scarcely viable infants with a birth weight of 1250—600 grams or less, and this conception has been accepted to a certain extent. For this reason and because in assembling the present material I only took the birth weight into consideration, I have retained the term premature, using it to denote infants with a birth weight of 2500 grams or less.

Obstetricians, on the contrary, have often used the length of pregnancy from the beginning of the 28th week up to and including the end of the 37th week as the only criterion of prematurity. The disadvantage is that the date of conception cannot be stated with certainty. Moreover, the statements regarding the date of the last menstrual period are often approximative and, in some cases, deliberately inaccurate.

As far as the length at birth is concerned, different examiners may give fairly divergent figures for the same children, on the grounds of such factors

[1] At Nordisk Pediatrisk Kongress, Helsinki 1946.

as the flaccidity of the tissues. It is therefore considered to lack reliability as a criterion of prematurity in scientific work.

The obvious advantage of using the birth weight is that, even with the type of scales that have been in use for many years, it can be given to within 10 grams. The disadvantage — which is seldom pointed out in the literature — is that weighing does not always take place immediately after birth but up to 30 minutes later. This applies in particular to multiple births and to complicated labour; the mother then requires immediate attention and weighing is postponed. During this time the infant may have urinated or evacuated the contents of the ampulla of the rectum. Asphyctic infants with meconium in the liquor amnii also have a somewhat too low official birth weight. For these reasons, the minimum weight might be a better criterion for comparative purposes, but this would necessitate standardized conditions in other respects during the first three days of life, which is scarcely the case.

The figure noted on the obstetric case-sheet as the birth weight is that used as the criterion of prematurity in all modern paediatric literature; this applies in the present paper as well.

CHAPTER II

Review of the Literature

1. Course of the Discussion

The prognosis for premature infants and the importance of prematurity for the occurrence of subsequent mental subnormality has been discussed by orthopaedists, psychiatrists, neurologists, obstetricians, paediatricians and psychologists. According to Wall, RITTER (1849) was the first in the medical literature to associate mental deficiency with high-grade prematurity. In 1862, LITTLE's work "On the Influence of Abnormal Parturition, Difficult Labours, Premature Birth and Asphyxia Neonatorum on the Mental and Physical Condition of the Child, Especially in Relation to Deformities" was published. Of the 63 cases in his series, 29 appear to have been premature (Brander). During recent decades, YLPPÖ 1919, CAPPER 1928, SARWAN 1930, BRANDER 1936, BENTON 1940 and DUNHAM 1948 in particular have made extensive surveys and criticisms of the relevant literature.

On broad lines, the course of the discussion has been the following. During the later years of the 19th century, intermittent reports were published of a relatively high incidence of low birth weight and birth before calculated term in cases of Little's disease, epilepsy and mental deficiency. In the beginning of the present century, up to about 1919, the prognosis for premature infants was discussed mainly by obstetricians. They based their statements on follow-up examinations of cases of complicated delivery. Induced labour before term was then a method frequently used. The results of the investigations showed that this method was associated with a considerable risk of immediate death of the child. The risk of later developmental disturbances was not considered to be so great. WALL's publication of 1913 is generally regarded as typical of this period.

YLPPÖ's now classical studies were published in 1919. They dealt mainly with the pathological anatomy, physiology, clinical aspects and physical development of the premature infant, but also touched briefly on the mental development up to school age. Ylppö's final prognosis on the last-mentioned point was somewhat pessimistic, particularly with regard to the smallest prematures. His opinion was strongly opposed to that of Wall and Pfaundler in particular, who had found no increased incidence of mental deficiency in their series. Ylppö stated that the high incidence of mental deficiency and of

spastic paralysis in his own series of prematures was to be ascribed to birt injuries and not to "degenerative" traits.

Between 1919 and 1927, only few articles were published on the prognosi for prematurely born children. They did not cover large series and contribute nothing essentially new to the discussion.

In 1927, COMBERG published, from the same maternity hospital as Ylppö a follow-up examination of premature infants born in the years following Worl War I. She included only children born in wedlock and belonging to relatively high social stratum and found the prognosis to be good. Only 1 ou of the 50 children was denoted as mentally deficient.

In the following year (1928), two reports were published which were o considerable importance for the further development of the discussion. LOOF in Norway was the first to make objective measurements of the mental devel opment of prematurely born children with the Binet-Simon-Terman test He used his own developmental test for children aged 1—3 years. He con cluded that prematurity is, directly or indirectly, an important causativ factor of mental deficiency and developmental disturbances in children CAPPER also reported on a series of prematurely born children in 1928. The were born in Vienna during World War I and the years immediately followin it. His final evaluation of the prognosis for these children was extremel pessimistic.

These investigations with their conflicting results and attempts to approac the problem from new angles gave fresh impetus to the discussion. A con siderable number of articles were published up to 1930, including MOHR an BARTELME's first work (1930) and that of OSSELMAN (1930), in which the I.Q was used as a gauge of the degree of development. The period may be con sidered to be ended with the publication of the extensive study made b the Norwegian obstetrician SUNDE in collaboration with the school psychi atrist LOFTHUS. They used the I.Q. to evaluate the development of th prematurely born children and presented a comparative series satisfactor from the environmental point of view.

During the subsequent few years, no studies were published on the relevan problems. In 1934, two publications in the U.S.A. re-opened the discussion The main conclusion drawn by ROSANOFF and INMAN-KANE from their in vestigation was that prematurity is an aetiological factor in the occurrenc of mental deficiency. They stated, however, that it is usually caused by birt injuries, such injuries being more common in premature than in full-ter infants. The publication of MOHR and BARTELME is still the most importan in this field. The study included both developmental and int test of children followed up for a considerable period. Moreover, the comparativ material is satisfactory from most points of view, since it consisted

term siblings of a number of prematures in the series. The authors found no reliable difference between the respective categories of children, if the number of months of prematurity was subtracted from the chronological age. Children who had suffered birth injuries showed a tendency to retarded development. The authors found no direct relationship between the I.Q. and the birth weight.

Two extensive studies were also published in Europe, namely by the Dutch obstetrician DUYZING (1935) and BRANDER (1936), from Ylppö's clinic in Finland. The results were conflicting. The former author concluded that prematurity in itself does not lead to mental deficiency, which is caused by genetic factors. Brander showed a linear relationship between the birth weight and the I. Q., after children with hereditary mental traits and severe birth injuries had been excluded. He expressed a pessimistic view with regard to the possibility of normal development in the smallest prematures.

The four last-mentioned publications dominated the discussion during the later part of the nineteen-thirties and the studies published up to the outbreak of World War II contributed no essentially new features. The results ranged between the extremes represented by the opinions of Mohr and Bartelme and of Brander, respectively.

Extremely few studies on the late prognosis for premature infants were published during World War II. After 1945, the discussion was re-opened; since then the conclusions have continued to range between the two extreme views already mentioned. A survey of the literature is given in tabular form.

2. Tabular Survey

Comment

Whenever possible, I calculated the number of those traced as a percentage of the number of prematurely born infants discharged alive from the hospitals in question. In some investigations, the number traced was not the same as the number who underwent a follow-up examination.

The methods used for the evaluation of the prognosis are abbreviated as follows:

Questionnaire to the mother	QM
Progress at school	PS
Clinical impression	CI
Intelligence quotient	IQ
Developmental quotient	DQ
Control group	CG

The pathological conditions frequently stated in the literature to be associated with premature birth are abbreviated as follows:

Yr. of publ.	Author	Country	No. of cases	Criterion of prematurity	Per cent traced	Age at exam. yrs.	Method used	Severe abnormalities reported
1913	WALL, M.	Germany	56	<9 mths.	37	2—20	QM PS CI	2 Ep
1919	YLPPÖ, A.	Germany	323	<2 500 Gm.	89 ½	½—8	QM CI	7.4 % Imb 3.1 % Sp
1921	SCHWARTZ *et al.* ...	U. S. A.	26	≦2 500 Gm.	—	1—6	QM	2 Dp
1923	LOOFT, C.	Norway	38	<9 mths.	—	¼—3 ¼	DQ	—
1924	BRANDT, P.....	Germany	72	<2 500 Gm.	84	7—?	CI	3 Sp 6 Dp
1924	FORSCHNER-BÖKE..	Germany	14	<2 500 Gm.	67	1 ½—7 ½	CI	1 Dp 1 Imb
1926	SALOMONSEN, L....	Norway	39	<2 500 Gm.	61	2—10 ½	CI	21 % Dp
1927	COMBERG, M.	Germany	73	<2 500 Gm.	89 ½	3—7	QM CI	1 Dp 2 Sp
1928	CAPPER, A.	Austria	103	<2 500 Gm.	58	1—19	CI	5 % Ep 7 % Imb 67 % Dp
1928	FRANKE, *et al.*	Austria	74	≦2 500 Gm.	43	4—10	CI QM PS	1 Sp 5 Dp
1928	KELLER, C.....	Germany	36	<9 mths.	87 ½	3—11	CI QM	7.5 % Sp 33 % Dp
1928	KORTHAUER, O. ...	Germany	81	≦2 500 Gm.	86	1 ¼—20	QM CI	28.5 % Dp
1928	LEVY, S.	Germany	77	<2 500 Gm.	46	1—10	CI PS	3 Imb 13 Sp
1928	LOOFT, C.	Norway	91	≦2 500 Gm.	—	7—?	IQ	7.7 % Dp
1929	HERZ, O.....	Germany	62	≦2 500 Gm.	74	1—5	CI QM	4.8 % Imb
1929	STEINFORTH, T. ...	Germany	73	≦2 500 Gm.	62 ½	7	CI	1 Sp
1929	ZIMMERMANN, I....	Austria	86	<2 500 Gm.	—	3—5	CI	5.6 % Dp
1930	RANKE, S.	Sweden	73	≦2 500 Gm.	67 ½	3—12	CI	5.5 % Dp
1930	SUNDE *et al.*	Norway	559	<2 500 Gm.	64	6—21	QM CI PS IQ CG	5.7 % Dp
1934	MOHR *et al.*	U. S. A.	250	≦2 500 Gm.	—	1—9 ½	CI IQ CG	—
1934	ROSANOFF *et al.* ...	U. S. A.	146	≦2 500 Gm.	42	6—16	IQ	10.3 % Dp
1935	DUYZING, A.	Holland	712	<38 wks.	95 ½	7—26	PS	6.6 % Dp
1935	FRIEDLÄNDER, A...	Denmark	64	<2 500 Gm.	67 ½	5—15	QM CI	30 % Dp
1936	BRANDER, T.....	Finland	376	≦2 500 Gm.	77	7—15	IQ	24.5 % Dp 8 Ep
1936	VON SYDOW, G.....	Sweden	37	≦2 500 Gm.	92	7—9 ½	IQ CG	7.1 % Dp
1937	BAEDORF, K.	Germany	27	≦1 700 Gm.	—	5 ½—17	QM PS IQ	5 Dp
1937	SIEDENTOPF, H....	Germany	188	<2 500 Gm.	—	10—14	QM PS IQ	14.7 % Dp
1938	VON CANSTEIN, H...	Germany	25	1 700—2 500 Gm.	67	9—16	QM CI IQ	1 Dp 1 Sp 1 Im
1938	SCHÖBERLEIN, W...	Germany	96	≦2 500 Gm.	35	6—18	QM PS IQ	3 Dp 3 Imb.
1938	SHIRLEY, M.	U. S. A.	63	<5 lbs.	—	½—2 ½	DQ CG	—
1939	BEITEL, L.....	Austria	119	≦2 500 Gm.	—	¾—6 ½	DQ IQ	—
1939	KERNAU, T.....	Austria	72	≦2 500 Gm.	45	2 ½—20	CI	6 Dp 2 Sp
1939	MUHL, G.....	Sweden	79	<2 500 Gm.	79	1—4	CI	3 Dp 3 Sp
1939	SCHULTZE, K.....	Germany	160	≦2 500 Gm.	42	8—13	QM CI	2 Imb 2 Dp 3 Sp
1941	EFFKEMAN *et al.* ..	Germany	29	35—47 cm.	64	6—10	CI PS	7 % Dp
1945	BARLOW, A.	England	514	<5 ½ lbs.	—	½—8	IQ CG	33.6 % Dp
1946	ASHER, C.	England	217	≦2 500 Gm.	32	1—6	DQ IQ	—
1948	DRILLIEN, C.....	England	103	≦2 500 Gm.	40	1 ½—4 ½	CI	—
1949	BESKOW, B.	Sweden	273	≦2 500 Gm.	92	9—17	PS IQ CG	12 Dp 1 Imb 2 Ep 9 Sp
1949	CHMELO *et al.*	Czechoslovakia	35	≦2 500 Gm.	12	2—14	QM	—
1949	HESS *et al.*	U. S. A.	216	≦1 260 Gm.	96	½—25 ½	CI	5.3 % Dp
1950	KAHL, M.	Germany	49	<1 700 Gm.	37	9—19	IQ	7 Dp 1 Sp
1950	KOENIG, H.....	U. S. A.	650	≦2 500 Gm.	—	2—10	CI IQ	4 Mo 10 Imb 2 Sp
1950	SCHACHTER, M....	France	183	≦2 500 Gm.	—	—	IQ	31 % Dp
1951	BRUSA *et al.*	Italy	543	≦2 500 Gm.	—	4—14	CI	31 Dp 3 Imb
1951	LEVESQUE *et al.* ...	France	60	—	50	3—?	IQ	8 Dp 3 Imb
1952	HOWARD *et al.*	U. S. A.	22	≦1 820 Gm.	—	8—19	IQ	—
1952	BLEGEN, S.....	Norway	541	≦2 500 Gm.	79	8—17	IQ	7.5 % Dp
1952	AHNSJÖ, S..	Sweden	374	≦2 500 Gm.	—	1—15	DQ IQ CG	—

Epilepsy	Ep
Spastic paralysis (Little's disease)	Sp
Severe mental deficiency	Imb
Slight mental deficiency ("Debilitas psychica")	Dp
Mongolism	Mo

It may be mentioned that the opinions regarding the borderline between severe and slight mental deficiency have varied appreciably during the period in question and in different countries. The present survey cannot, therefore, be considered as exact in every detail. Its object is merely to give a brief review of the way in which attempts have been made by various authors to evaluate the late prognosis for prematurely born children.

3. Critical Survey of Certain Investigations

A more detailed account is given in the following of some investigations which are of general interest and which are based on relatively large series. In the majority of instances, the follow-up series consisted of more than 100 cases. It is obviously extremely difficult to judge these investigations fairly in the light of the knowledge of the relevant questions prevailing at the time of publication. I have therefore judged them according to the present views on how a series of prematurely born children should be compiled.

My object in making this more detailed, critical survey of the literature was to attempt to ascertain the reasons for which the results have not withstood the proof of time. The views on the long-term prognosis for prematurely born children are still extremely divergent. I have focused particular attention on those factors which make it inadvisable to draw generally applicable conclusions from the series in question. In certain cases I have also, on the basis of the primary figures given, made re-calculations of certain factors in order to make it easier to survey these studies against the background of my own problems.

With regard to the intelligence and developmental tests used in the various investigations, I have retained the denotations used by the authors and have not attempted to introduce any uniform nomenclature.

WALL's (1913) basic material consisted of 691 prematurely born children of mothers who had been in-patients or out-patients at the Breslau Obstetric Clinic in 1892—1910. Of these children, 492 survived the first weeks of life. Wall was able to trace 200 of them and to obtain information about 183 (37.2 per cent); 126 were dead, and 57 alive and between the age of 2 and 20 years. The criterion of prematurity appears to have been a pregnancy of less than 9 months. The initial social and economic status was poor. The follow-up

investigation was based on a questionnaire addressed to the mother; in those cases in which a personal examination was made, the clinical evaluation of the author was given. A report of the achievements at school was given for the children of school age.

According to Wall, these children walked and talked at a later age than that considered to be normal, the retardation being directly proportional to the degree of prematurity. The schoolchildren all attended ordinary schools and appeared to have compensated for the slight retardation shown in preschool age. Two children were epileptic, but none of those in the series suffered from spastic paralysis or were mentally deficient. A number exhibited nervous symptoms such as nocturnal enuresis, speech disorders or night terrors. The objections that may be raised to this investigation are that the incidence of those traced was low, that many of the data were based on the mother's statements alone and that no normal material, comparable from an environmental point of view, was presented. The actual number of cases followed up was exceedingly small and the author's subjective evaluation seems to have been based on a single examination.

Wall's work appears to have had considerable influence on the opinions regarding the prognosis for premature infants during the years following its publication. It was frequently quoted and contrasted to Ylppö's results with respect to the late prognosis.

Ylppö's (1919) series consisted of 668 premature infants born at Kaiserin Augusta Viktoria Haus between 1910 and 1918. He followed them until the age of 8 years and obtained information about all except 70. These 70 consisted mainly of older children, so that 28 per cent of the 7-year-olds and 21 per cent of the 8-year-olds could not be traced. Of the remaining 598, 320 were dead. Ylppö gave the incidence of idiocy or of definite imbecility as 24 (7.4 per cent) and of Little's disease as 10 (3.1 per cent). With regard to length and weight, he stated that the premature infants with a birth weight of over 1000 grams reached the same level as the full-term infants between 5 and 6 years of age — "the age of conception" as he termed it.

In my opinion, the objections that can be raised to this study as a basis for generally applicable conclusions regarding the prognosis for premature infants are — on broad lines — the following. The expectant mothers, and subsequently their children, lived in Berlin under the conditions of starvation and hardship that prevailed during World War I and the years immediately following it. There was a high incidence of rickets among the children and epidemic influenza raged during several years. The incidence of premature births was particularly low, *i.e.*, 5.3 per cent as compared to 10—11 per cent at other maternity hospitals in Germany during the same period. Ylppö himself stated on page 8: "Diese Zahl kann aber keineswegs für die deutschen

Verhältnisse als Norm betrachtet werden, denn in unserer Entbindungs-abteilung werden nur Frauen aufgenommen, die bei der vorausgehenden Untersuchung als gesund befunden werden und in Bezug auf den Geburtsverlauf keine Komplikationen erwarten lassen."[1] The premature infants who were born at this clinic had, according to Ylppö himself, a good initial situation from a medical and obstetric point of view in comparison to the current norms for Germany as a whole.

COMBERG's study (1927) was from the same clinic as Ylppö's. It covered the premature infants born between 1919 and 1922, but only included the children born in wedlock and belonging to educated and financially secure social groups. The author stated that children born out of wedlock comprised only a small part of the material at this clinic. The infants in her series were either born at the clinic or had been transferred there. The latter category may be considered to be selected in that no dead children were represented, whereas the living children with complications were presumably over-represented. This can, however, only be surmised since no such data are given. The series originally consisted of 233 children; 136 of them died during the first year of life. Of the remaining 97, 81 could be traced; 50 of them underwent a follow-up examination between the age of 3 and 7 years. Written information was obtained about a further 23. Only one out of the 50 children examined could be denoted as mentally deficient. A further two suffered from Little's disease. Comberg considered the prognosis for prematurely born children to be definitely good, although there was a high incidence of nervous symptoms such as irritability, hyperactivity, negativism, anxiety and disturbances of sleep.

CAPPER (1928) published in the U.S.A. a report of a series from the Paediatric Clinic of the University of Vienna. He denoted the children with a birth weight below 2500 grams as immature and those born before calculated term, irrespective of the weight, as premature. Between 1911 and 1926, 247 children with a birth weight definitely below 2500 grams were discharged from the clinic. Reliable statements regarding 140 of them could be obtained; 23 had died and 103 returned for a follow-up examination. Capper examined them himself and included information concerning their achievements at school. The age at the follow-up examination ranged between 1 and 19 $^1/_2$ years. The oldest of them could not have been among those born in 1911—1917, since the examination was presumably made in 1927. His conclusions were definitely pessimistic: "almost one half of them die during the first year

[1] These figures cannot, however, in any way be considered as a norm for conditions in Germany, since only those women were admitted to our obstetric department who were found at a preliminary examination to be healthy, and no complications were expected in the course of labour.

of life. Of those that remain alive, the majority are physically as well as mentally underdeveloped . . . In brief, the immature infant becomes the backward school child, and is a potential psychopatic or neuropathic patient and even a potential candidate for the home for imbeciles and idiots."

The main drawback to Capper's series is that it consisted principally of children born during a time of war or depression, when the population was in an exceptionally poor nutritional state, and of those from socially and economically badly situated groups. Moreover, no comparative material representative of these conditions was presented.

LOOFT in Norway published reports in 1923 and 1928 of the developmental and intelligence quotients of prematurely born children. In 1923 he gave an account of 38 children aged 3—38 months, all of whom showed signs of rickets. He used his own developmental test as the norm and made comparisons with full-term infants, both those who had had rickets and those who had not had this illness. He found that 75 per cent of the prematures were retarded as compared to the healthy full-term infants, but not in comparison to those who had rickets. He concluded that the retarded development could mainly be ascribed to rickets and that it should be transient.

In 1928, Looft reported on several additional series:

a. 91 children, aged $4\,^1/_2$—$11\,^1/_2$ years, with a birth weight of 2500 grams or less. They were tested with the Binet-Simon-Terman test; 7 had an I.Q. ranging between 57 and 87.

b. 29 prematurely born children delivered in their home; the I.Q. ranged between 43 and 88.

c. 532 children of school age denoted as mentally deficient; in 29 cases Looft considered the cause to be prematurity. In 19 cases the I.Q. ranged between 71 and 88.

d. 15 prematurely born children who had just started school; 1 of them was mentally subnormal.

e. 105 prematurely born children from Looft's private practice as a child psychiatrist. 10 of them had intellectual disturbances only; 19 had Little's disease and 3 epilepsy, in every case accompanied by mental disturbances.

f. 150 children in an institution for the mentally deficient; prematurity was stated to be the cause in 8 cases. In other such institutions, the corresponding figure was 27 out of 717.

No generally applicable conclusions can be drawn from these series, which in the majority of cases were selected in a negative direction, since they comprised children who had been brought to a child psychiatrist, or who had been institutionalized for mental disturbances. No representative comparative figures are given. A merit of the investigation is the introduction of more objective standards of evaluation.

Another investigation from Norway was published in 1930, that of the obstetrician SUNDE in collaboration with the school psychiatrist LOFTHUS. Their original material consisted of 1143 premature infants discharged alive from a maternity hospital. Four hundred and fourteen of them could not be traced, *i.e.*, 64 per cent were traced. The follow-up examination was made on 559 prematurely born children aged 6—21 years, the majority being between 10 and 15 years of age. At the time of the investigation, 109 of them attended school in Oslo; 102 were tested with the Binet-Simon-Stanford test. The comparative material consisted of 150 children from a school in Oslo selected at random; they underwent the same tests. The mean I.Q. in the control series was 94.5 for the boys and 92 for the girls; the corresponding figures for those born prematurely were 85 and 83. The authors concluded that the intellectual development of the prematurely born children was retarded in comparison to that of the children in the normal material. No direct relationship could be demonstrated between the I.Q. and the birth weight.

The main fault that can be found with this investigation is that more than one-third of the children could not be traced. In addition, no data are given regarding the social and economic background of the two series, except that the form of schooling was the same in both cases. The great advantage is that, for the first time in such an investigation, a representative comparative series was presented.

MOHR and BARTELME's first study on the mental and physical development of premature infants was also published in 1930. It was subsequently enlarged and published in its final form in HESS, MOHR and BARTELME's book of 1934. The 1930 investigation comprised 113 prematurely born children compared with 40 siblings of some of these infants. The age of the prematurely born children ranged between 8 months and 7 years, the mean age being 2 years 9 months. The mean age of the siblings was 5 years 8 months. Two tests were used: the Gesell Developmental Schedule for 107 children less than 5 years old, and the Kuhlman-Binet Scale for 113 children over 18 months of age. In both cases the chronological age was corrected for prematurity. The later investigation comprised 250 children selected at random among those attending an out-patient clinic for prematurely born children. The controls consisted of 152 full-term siblings from 108 families with premature infants. Their age ranged between 1 and 9 $^1/_2$ years. The children were from a slightly lower social and economic group than the norm. The follow-up examination was made in the same way as in the 1930 investigation, except that the I.Q. of all the children was recorded and the Gesell Schedule used for all those below 6 years of age.

The authors concluded that, if the degree of prematurity was taken into consideration, there was no reliable difference between the prematurely

born children and their siblings with a normal birth weight. This also applied in a comparison with the norms for the tests. The Gesell Schedule showed a tendency to retardation during the first two years of life, if the chronological age was not corrected. No statistically significant correlation was present between the birth weight and the I.Q. Nor was any statistically significant difference found between the children of healthy mothers and of those who suffered from toxaemia, renal complications or syphilis during pregnancy. A tendency to retarded development was found in 69 children who had presumably suffered slight brain damage during birth. There was a higher incidence of nervous symptoms among the prematurely born children than among their full-term siblings.

The merits of this extensive investigation are obvious. The social and economic factors were taken into consideration by means of satisfactory comparative material. Objective methods were used for the tests. The children were under observation for a long period and were familiar with both the examiners and the surroundings.

The objections that can be raised are negligible in comparison to the merits. For example, the series was stated to be "selected *for the most part* at random" but no details are given. The clientele at a follow-up clinic for prematures usually consists of children who have suffered birth injuries and of those whose mothers are ambitious, in particular when the child in question is their first child or first premature child. The number of premature children born out of wedlock is, at any rate according to Swedish standards, extremely low, *i.e.*, 8 out of 250. Five-sixths of the children were followed up only until they were of school age.

In 1934, another study was published in the U.S.A., that of Rosanoff and Inman-Kane. Among other matters, they investigated the number of prematurely born children among mentally defectives as compared to an unselected population. They found an incidence of 21.31 per cent among 122 in the former category, as compared to 3.89 per cent in a normal population of 9 782 persons. They also investigated the incidence of mental deficiency among prematures as compared to an unselected population. Of 347 prematures discharged from the maternity department, 146 could be traced. In 140 cases intelligence tests were made; 10.27 per cent were found to have an I.Q. below 75, 17.9 per cent below 85 and 19.3 per cent above 115. The median was 100. In Terman's group of 905 unselected children, 11.55 per cent had an I.Q. above 115. Rosanoff and Inman-Kane concluded that prematurity is an aetiological factor in mental deficiency, although in the majority of cases it is due to birth injuries, which are more common among prematures.

The criticisms that may be made of this study are mainly that only 42 per

cent of the children could be traced and that data concerning the social, economic and genetic background are lacking.

In 1935, the Dutch obstetrician DUYZING reported a follow-up study of 712 prematurely born children, selected according to the length of pregnancy without consideration to the birth weight. The children were born during the 28th up to and including the 37th week of pregnancy and a number of them weighed over 3000 grams at birth. The age at the follow-up examination ranged between 7 and 26 years. A calculation of how many of the 1450 liveborn infants survived the 10th day of life gives the figure 1145; 340 appear to have died later and 712 were examined, *i.e.*, approximately 92 per cent were traced. The follow-up examination consisted of a written questionnaire, personal examination, statements regarding the achievements at school and, in the few cases over 20 years of age, regarding military service. Of these 712, 42 were considered to be mentally subnormal and 5 imbecile, *i.e.*, 6.6 per cent were mentally deficient. As a comparison, 2 per cent is given as the mean figure for the German population. In the 47 cases of mental deficiency, hereditary traits were considered to be present in 33 cases, to be absent in 9 cases and in 5 cases the genetic investigations were not complete. The authors concluded that although mental deficiency is more common with a low birth weight and shorter pregnancy, prematurity in itself is of little importance but that genetic factors play the decisive role.

The main objections that can be raised to this extensive study are the following. Primary figures are seldom given for calculations of the incidence. No comparative material typical of the social class and environment is presented. The series includes an unspecified number of children with a birth weight ranging between 2500 grams up to over 3000 grams. The data regarding hereditary traits and other genetic factors must be considered as insufficient. A stringent genetic analysis must be made far more thoroughly.

A preliminary report was given by BRANDER in 1935; it was published in its definite form in 1936. The series comprised 376 prematurely born schoolchildren aged from 7 to 15 years. Of the 1070 who had been discharged alive, 240 had died later and 583 could not be traced, *i.e.*, 77 per cent were traced. The Binet-Simon-Terman test was made in 376 of these cases. The social and economic background of the children must be denoted as exceedingly poor. The mean I.Q. was approximately 85. When those prematures with hereditary traits, complications during delivery or both these factors were excluded, 230 children remained; it was found that the lower the birth weight, the lower was the I.Q. Brander stated that he found a rectilinear relationship between the I.Q. and the birth weight, so that a difference of 500 grams in the birth weight corresponded to 7—8 units in the I.Q.

Much attention was accorded to Brander's study and in all the subsequent

discussions it has been contrasted to the findings of Mohr and Bartelme. The questionable features are that the social and economic situation of the children was poor and that the material was selected to some extent, in that there was a high incidence among the parents of alcohol addicts and of unmarried mothers. Moreover, a control series was lacking and 23 per cent of those who were discharged alive from the maternity department could not be traced.

Of the publications later than Brander's, I shall only discuss those in which the follow-up series consisted of more than 100 cases and those which, despite a smaller series, made some new contribution to the discussion.

SIEDENTOPF (1937) gave an account of a follow-up examination of 188 prematurely born children; only a relatively small percentage could be traced of those who had left the maternity department alive. The age at the examination ranged between 10 and 14 years. A personal examination was made in 100 cases; in the remainder information was obtained from replies to a written questionnaire. The mental development was assessed mainly on the school reports, which were considered to be a better basis than the I.Q. on a single occasion. The achievements were denoted as good in 34.6 per cent, moderately good in 46.3 per cent and poor in 19.1 per cent; 14.3 per cent of the last-mentioned category were unable to follow the ordinary instruction at school. A comparison was also made between 100 prematures born in wedlock and 88 born out of wedlock. In the former category there were 46 per cent good pupils and 15 per cent poor pupils; the corresponding figures for the children born out of wedlock were 21.6 per cent and 23.9 per cent.

The objections that may be raised to this investigation are that no objective methods were used for the testing and that no normal figures were given. The comparison between those born in and out of wedlock, respectively, is of interest but the series are relatively small. Nor were any normal figures given for this part of the investigation.

SCHÖBERLEIN published in 1938 a study of premature infants all born in a maternity hospital and in wedlock; the age at the follow-up examination was between 6 and 18 years. Altogether 275 such infants were discharged from the hospital but only 96 could be traced. Almost half of them weighed less than 1 500 grams at birth. The follow-up examination was made by means of questionnaires addressed to the parents and teachers and Binet-Simon-Terman tests.

The author concluded that the I.Q. rises with an increase in the birth weight. Of those examined, 3 were mentally subnormal and 3 imbecile, *i.e.*, altogether 6.3 per cent of the whole material. According to the teachers, only those children with a birth weight of more than 1 500 grams were above the normal intellectual level. The mothers described the children, particularly those with a very low birth weight, as irritable and nervous.

In this study, the percentage of children that could not be traced was remarkably high, and no representative control series was described.

BEITEL reported in 1939 on a follow-up examination of 119 prematurely born children. The age ranged between 0.8 and 6.5 years, the majority being between 2 and 4 years old. In order to avoid the influence of hospitalism, those who had latterly been cared for in institutions were not included. From an economic and social point of view, the series was on a fairly high level. Fifty per cent of the children came from working-class homes and 4 per cent were born out of wedlock; 97 per cent lived at home. The examiner was not previously acquainted with the majority of the children but good contact was stated to have been established in 80 per cent of the cases and no difficulty in carrying out the examination was encountered in the remaining cases. The Bühler-Hetzer test was used; 12.5 per cent were found to be above the normal level and 29 per cent below. Beitel also divided her series into other groups in order to permit comparisons in tabular form with the results of both Mohr and Bartelme and of Brander. The author summarized her views in the statement that after the age of three years the I.Q. of the prematurely born children corresponds to the norm for the test. She found only a slight relationship between the I.Q. and the birth weight. Beitel stated that she found a relatively more rapid mental development during the first years of life; in this respect her views agree with those of Gesell.

The main criticism that can be made of this investigation is that the children were followed up for only a short period and that no representative comparative series was presented.

In 1939, SCHULZE published an account of a follow-up examination of 160 prematurely born children; they comprised those who could be traced out of the 382 who were discharged alive from the maternity department. At the time of the examination, the age ranged from 8 to 13 years. A personal examination was made in 97 cases and information about 63 was obtained from a written questionnaire. A comparison was made with their siblings with respect to weight, height and general impression. Forty per cent of both the prematurely born children and their siblings were below the normal physical and mental level. Cerebral disorders were present in 4.8 per cent; this included 1 case of mongolism and 1 of microcephaly.

In this investigation, the number traced was somewhat small and no objective methods of examination were used.

The first large investigation after World War II was published in 1945 in England by BARLOW. The follow-up examination was made on a series of 514 prematurely born children; 255 of them were taken from the out-patient material of the hospital and 259 were private patients. The control series consisted of 149 full-term children from the out-patients' department and 352

full-term private patients. Mental abnormalities of some kind were found in 33.6 per cent of the prematures, the corresponding figure for the controls being 11.2 per cent. When both series were divided into four weight groups, the percentage deviations remained unchanged in all the groups in the control series, but rose from 31.2 per cent to 64 per cent with a decrease in the birth weight in all four groups of prematures. Between 6 months and 3 years of age, 42.6 per cent of the 244 prematurely born children were "abnormal" as compared to 19 per cent of the 174 controls. Of the total 173 prematures denoted as "abnormal," 11 were mongols and 17 had various disorders, some of which must be considered as a cause of prematurity rather than as a result of it.

The objections that may be raised to this investigation are those most frequently occurring, *i.e.*, that the material is selected and that no information is given concerning the social and economic background of either the prematurely born children or the controls. In the former category, 50 per cent derived from the out-patients' department of the hospital, whereas in the latter category the figure is only 30 per cent. The remaining 50 per cent and 70 per cent, respectively, were denoted as "private patients" but no account was given of the social and economic implications. At the follow-up examination, 47.5 per cent of the prematurely born children were from 6 months to 3 years of age; the corresponding figure for the controls is 34.7 per cent. Even those authors who are optimistic regarding the prognosis for prematures agree that the prematurely born children have not reached the level of the full-term infants by this time. In addition, as in the majority of investigations, Barlow did not distinguish between those pathological conditions that may cause prematurity and those that may be a result of it.

In 1946, another report was published in England. ASHER planned a follow-up study of 205 prematurely born children who were discharged alive from a children's hospital. Only 58 of them could be traced and examined and a further 8 were found to have died. Thus, information could be obtained about only 32 per cent. This was ascribed by the author to the constant change of residence during the war, particularly in the industrial areas. In order to increase her series, Asher included premature infants who had been admitted to the hospital on other grounds than prematurity or those who were registered at the child welfare centres. With these additions, her series consisted of 217 prematurely born children. Those under 3 years old were tested with the Gesell Developmental Schedule and those over 3 years with the Binet-Simon-Stanford test. The results were calculated with correction for the chronological age.

It was found that the prematurely born children below 3 years of age were 1—2 months retarded in their development, whereas the mean I.Q. for those

of 3—6 years old was 92—93. The author stated that there was a direct relationship between the birth weight and the I.Q. She concluded that there are four types of prematures: (1) those who reach the same level as full-term infants during their first year of life; (2) those who develop slowly but gradually attain the normal level; (3) those who are mentally subnormal and thus never reach the normal level; (4) those with Little's disease or other nervous motor disorders.

It may be objected that the percentage traced was low, but this applies to all similar investigations made after 1945 in the European countries involved in World War II. Asher's complementary material was presumably selected to some extent, since it was taken from hospitals or from institutions for prophylactic child welfare. Moreover, her material derived from a large manufacturing town with its particular social and economic problems.

In 1948, DRILLIEN published a report on 103 prematurely born children compared with 174 full-term children from the same hospital. She sent questionnaires to the parents of 285 prematures and 430 full-term children, selected at random 1 out of every 10. Replies were received in approximately 40 per cent of the cases but it was not possible to ascertain the number of deaths. The children were followed only until the age of $1\,^1/_2$ to $4\,^1/_2$ years. The main interest was focused on the conditions during the first years of life. The children were not tested, but 15 per cent of the full-term infants and 23 per cent of the prematures were stated to exhibit behaviour disorders. Drillien expressed the opinion that the difference between the respective categories after the first years of life was dependent upon the environment, and that the same factors were operative post-natally as pre-natally.

The following points may be raised. The number of children traced was small, the observation period was short, and no objective tests were made of the mental development.

An extensive study was published in Sweden in 1949 by BESKOW. It consisted of follow-up examinations of 273 prematurely born children between the age of 9 and 17 years. The incidence of those traced was 92.1 per cent. The social and economic situation was denoted as extremely poor; 60 per cent were born out of wedlock. The home conditions were deplorable in 10 per cent of the cases and in 12 per cent the father was an alcohol addict. Scarcely 50 per cent of the children came from an "irreproachable environment." The Terman-Merrill test was used and the results compared with the mean figures for the schoolmates. The children were tested at intervals of one year and the results analyzed to exclude pseudo-oligophrenia on the grounds of speech retardation or poor motor development. Twenty-five per cent of these children had been referred to a child guidance clinic for difficulties at school (difficulties in following the instruction in 49 cases, reading disability in 3

and pronounced nervous disturbances in 16 cases). The I.Q. was below 85 in 16.11 per cent of all the prematurely born children and below 60 in 4.76 per cent; one child was considered to be ineducable. There was a definite history of a cerebral haemorrhage in 6.59 per cent of the cases. If these children and those with mentally abnormal parents are sorted out from those with an I.Q. below 85, this group had a somewhat lower I.Q. than the remaining children, *i.e.*, 2 units for the boys and 4 units for the girls. Beskow was unable to confirm Brander's observation of a direct relationship between the I.Q. and the birth weight, but found the I.Q. to be independent of the birth weight in the prematurely born children.

Epilepsy was present in 3 of the children and spastic paralysis in 9. These children comprised half of those who had been suspected to have had a cerebral haemorrhage at the maternity hospital. The incidence of manually unskilful and nervous children was extremely high. The children born in wedlock did better at school and had a lower incidence of nervous symptoms than those born out of wedlock.

In 1947, BESKOW published a study on the distribution of nervous symptoms in children during the first years of life. He gave an incidence of 32.5 per cent for children with a normal birth weight, whereas the corresponding figure for prematures was 66.6 per cent.

The advantages of Beskow's study are the high incidence of those traced and the thoroughness of the observations. A disadvantage which makes it difficult to draw any generally applicable conclusions is that the social and economic background of the children was exceptionally poor. Moreover, no control series typical of the same conditions was presented, but the comparisons were made with the mean figures for schoolchildren in general.

HESS, LUNDEEN, KUNDSTAEDTER and ENGLE reported in 1949 on 259 prematurely born children with a birth weight between 735 and 1 260 grams, who had been discharged alive from the Premature Station of the Sarah Morris Hospital in 1922—1947. Of those discharged, 33 had died later and 10 could not be traced, *i.e.*, 96 per cent could be traced, which is an exceptionally high figure for an investigation of this nature. A follow-up examination was made of 216 patients; 188 were white and 28 coloured. Twenty-four were 17 years old or above; 3 of them had completed their military service. The children were classified into five groups according to the physical and mental development, with the following results:

	Physical Development	Mental Development
Normal average — some superior	101	123
Slight deviation	68	54
Poor	20	21
Bad	20	6
Extremely subnormal	3	5
	212	209

Of the 59 children with a suspected cerebral haemorrhage, 28 showed no signs of residual defects, whereas in the remaining 31 cases the symptoms were slight in 1, moderate in 10 and severe in 20 cases.

In this investigation, the main interest was focused on the small prematures. An extremely high percentage was traced and the follow-up period covered many years. On the other hand, the test methods were somewhat approximative and no account was given of a comparable control series from the environmental and social point of view.

In 1950, KOENIG published a report of a series of 700 prematurely born children whom she had followed up by means of annual visits to the out-patients' department for prematures. Thus, 150 children with a birth weight below 2 kilograms and 500 between 2 and 2 $^1/_2$ kilograms were followed up until the age of 2—10 years. The material was also divided into sub-groups in order to ascertain whether there was any borderline below which no particularly high values were found. The children were found to have attained normal height and weight by the age of six years or even earlier. Intelligence tests were made in 28 cases selected at random (the children happened to visit the clinic on those days on which the psychologist was in attendance). Using the Binet-Simon tests, the I.Q. ranged between 83 and 124. An additional 99 children were tested with the Otis Alpha form A. The quotients ranged between 66 and 137; these were stated to be no lower than those of schoolchildren in the same town with the same background. Even those children with a birth weight below 2 kilograms obtained good results.

Koenig made no mention of how many children were discharged alive from the hospital. Nor did she state the percentage of children who returned for follow-up examinations nor whether there was a preponderance of any particular type of prematures among those attending. In addition, representative control material was lacking.

A paper by the Frenchman SCHACHTER was published in 1950 in an Italian journal. The report was based on the follow-up examination of a child weighing 860 grams at birth, but an account was also given of an examination of 183 prematurely born children brought for consultation on the grounds of mental disturbances. No mention was made of their age. On the basis of the Binet-Simon tests, 41 per cent were denoted as normal, 28 per cent as borderline cases and 31 per cent as definitely mentally subnormal.

Only a brief account is given of the series and no generally applicable conclusions can be drawn, since the investigation was confined to those who were brought for examination on the grounds of behaviour disorders or mental subnormality.

In 1951, BRUSA and MENGHI of Italy reported a study of 543 "immature" children (birth weight 2500 grams or less) followed up for 4—14 years. Rela-

tively few of them were small prematures, *i.e.*, only 10 weighed less than 1500 grams. The authors stated that the mortality was high during the first two years of life but that subsequently it was comparable to that among full-term infants. They found that the physical development soon reached normal standards. The mental development was normal in the majority of cases. They denoted 31 children as being moderately mentally deficient and 3 as imbeciles, but did not state the criteria used. The incidence of those born out of wedlock was 15.65 per cent. All the children were born or lived the first years of their life during World War II. No control series was studied. No general conclusions can be drawn from this investigation.

LEVESQUE and COFFIN in France published an account in 1951 of 95 personally examined prematurely born children. They received replies to 300 out of 600 questionnaires. "Troubles de caractère" of varying degrees were present in 46 cases. The I.Q. was determined in 60 children aged between 3 and 12; 24 were of normal intelligence or above, 25 were one year below the normal, 8 two years below and 3 were denoted as imbeciles.

Few inferences can be made from this study, since the number of cases missing is too large and no control series is reported.

In 1952, another investigation was published in Norway, namely that of BLEGEN. She reported on a large series, taking into consideration not only the immediate mortality but also the physical and mental prognosis up to and including school age. The mental examination covered 541 prematurely born children between the age of 8 and 17 years. They comprised those that could be traced out of the 893 liveborn children; 682 were presumably alive at the time of the investigation. Thus, only 79.3 per cent could be traced.

The children were tested with a group test during their first year at school. On this basis and the teacher's general evaluation, all those who appeared mentally retarded were picked out; after a special physical and psychological examination, they were assigned to a suitable form of schooling. According to the text, all those who attended an ordinary elementary school and did not require a special examination had an I.Q. above 80. According to the tables, however, they should have had an I.Q. of over 90. She found the following distribution:

I.Q.	>90	476 children (presumably a printer's error, since the figure should be 474)
I.Q.	81—90	15 »
I.Q.	71—80	27 »
I.Q.	51—70	18 »
I.Q.	36—50	4 »
I.Q.	35	3 »

Thus, 4.6 per cent had an I.Q. of 70 or less. A follow-up examination of the children attending the ordinary classes was made by means of a questionnaire addressed to the teachers. Blegen found that the social background was an extremely important factor and that the prematurely born children often had a poorer social and economic status, judged according to the housing conditions, than schoolchildren in general. She stated that an improvement in the social background and in the housing conditions would presumably result in better mental development.

The criticisms that may be made of this investigation are the following. The incidence of children traced was relatively low. No study was made of a control series of full-term children with a comparable social background. The use of the number of persons living in each room as the main basis for the social classification appears to be somewhat questionable, particularly in view of the post-war housing shortage. Even if the conditions referred to the pre-war years, it seems that a more satisfactory basis for the classification might have been chosen.

In 1952, HOWARD and WORRELL reported on a follow-up examination of 22 prematurely born children with a birth weight up to 1820 grams. The age at the examination ranged between 8 and 19 years and the main interest was focused on the development of the intelligence and personality. The physical development was stated to be normal in the majority of cases, although errors of refraction were more common than could be expected in normal children. Four different tests were used for the mental development. The resulting curve for the distribution had a normal appearance; two of the children had an I.Q. of 130 and 132, respectively, and two had an I.Q. of 55 and 56, respectively. Delivery had been difficult in 5 out of the six children with an I.Q. between 70 and 80. The authors concluded this to be an indication that prematurity does not affect the mental development. They found no correlation between the degree of prematurity and the intellectual capacity. They recommended that attendance at school of prematurely born children should be delayed for one year.

Several of the children appeared, owing to emotional maladjustment, to have difficulty in taking advantage of their possibilities. Two personality tests were used on 21 of the children and the results compared with the opinion of the parents. Five children had earlier been under the care of a psychiatrist for difficulties in adjustment. The authors concluded that the "emotional instability" noted was increased by the overprotective attitude of the parents, the initial poor physical development and possibly by "the rigid type of immediate postnatal care"; during this period these children lack the ability to express their wants.

The article presents many interesting features, but has the disadvantage of being based on a large number of examinations on a small series.

In a lecture given in 1952, AHNSJÖ reported on a series of prematures in Stockholm. It has hitherto only been published as a review by the author. The series consisted of 374 prematurely born children admitted between 1945 and 1951 to the Home of the Child Welfare Authorities of the City of Stockholm (Nybodahemmet). The age of the children at the examination ranged between 1 and 15 years. Although the series was relatively small, it was divided into twins and single births. It was then classified further into five groups according to the presence or absence of complications during delivery (K), hereditary factors (H) and environmental factors (M). No mention is made in the review of how the two last-mentioned factors were defined. The resulting groups were, for example, H–K–M–, or H–K–M+. A comparison was then made between each group of prematures and a control group, as far as possible identical, consisting of children born in the same year as the prematures and with a birth weight of more than 2 500 grams.

The mean values for the motor functions of the prematurely born children in group H–K–M– (for which the calculated length of prematurity was given) showed a retardation corresponding to the time the child was born before calculated term. The prematurely born children with a birth weight below 2000 grams, on the other hand, showed a greater retardation than that corresponding to the degree of prematurity.

In a smaller series, in which the Bühler-Hetzer and Terman-Merrill tests were used, the children with a low birth weight, in groups H–K–M– and H–K–M+ also showed, as a rule, I.Q.s within the normal limits. The majority of low I.Q.s were noted in children of pre-school age.

A higher incidence of nervous symptoms was also found in group H–K–M– of premature single births than in the group of premature twin births and the controls.

Drawbacks from a social and economic point of view are usually associated with a series consisting of institutionalized children, when it is to be used as a basis for more generalized conclusions. These have been compensated for in Ahnsjö's study by the use of a control series comparable from the majority of aspects. Since severely mentally deficient or crippled children are usually placed in institutions appropriate for their care, very few of these cases can have been included. It may, however, be presumed that in the majority of cases such children would have belonged to the groups with H+ or M+. In this case, they would not have been assigned to groups H–K–M– or H–K–M+ discussed in the aforegoing.

4. Summary

In order to summarize the literature and to record the aspects of the relevant problems on which opinions are unanimous and those on which opinions are at variance, I have drawn up the following points.

It is generally agreed that:

1. The physical and mental development of prematurely born children is retarded during the first two to three years of life. This retardation is most marked in children with a particularly low birth weight. If the time before calculated term at which the child is born is taken into consideration, and a corrected chronological age is used, the retardation is less marked.

2. In the majority of investigations on the prognosis for premature infants, the incidence of severe mental disturbances is stated to be relatively high. Only a few studies have failed to report a high incidence. According to a survey of 19 articles made by PEIPER (1937), such disturbances are found in 5.5 per cent of prematurely born children.

3. As early as the beginning of the present century, nervous disturbances were stated to be more common in prematurely born children than in those born at term. These symptoms may be psychosomatic (*e.g.* increased fatigability, disturbances of sleep), emotional (*e.g.* anxiety, shyness, irritability, emotional outbursts) or intellectual (*e.g.* short span of attention, poor memory, lack of concentration).

4. Prematurely born children are more often motorially clumsy and relatively often exhibit speech disorders or reading disability.

The points on which opinions are extremely divergent are mainly the following.

1. At what time is this retarded development, both physical and mental, compensated and is it ever compensated, even in children who have suffered no birth injuries?

2. Is there any direct relationship between the birth weight and the mental development and, if this is the case, how strong is the correlation?

The answers given to these questions by the various investigations are, in some cases, diametrically opposed. Moreover, practically all the shades of opinion between these two extremes are represented in the literature.

The question arises of the reasons for these discrepancies. As far as I have been able to ascertain, the main reasons are the following.

a. For more than thirty years, prematurity has been defined by means of a borderline figure partly dependent upon the decimal system. The introduction of this borderline figure constituted a considerable advance, since still more vague conceptions had been in use earlier. The investigations of prematurely born children published during the past 20 years have given increasing

evidence of the heterogeneity of the term prematurity. Actually, the birth weight is affected by *genetic* factors such as descent from various types of isolates. It is, for example, possible that the mean figure for the birth weight varies for different races. It has also been shown that, in certain families, all the children have a low birth weight on no demonstrable medical grounds. There is also a definite difference between the birth weight of boys and girls. The birth weight is also affected by *social* and *economic* factors. These may result in a difference in the nutritional state of the mother during pregnancy. In addition, such factors as heavy work until parturition and varying degrees of mental stress are present in some cases. Only in half of the cases is there an established medical cause of the low birth weight. Of the *obstetric* factors, the most common are presumably various forms of proteinuria, haemorrhages, acute infections, syphilis, endocrine disturbances and purely mechanical obstetric abnormalities and multiple births. *Paediatric* factors, such as malformations and mongolism, also affect the birth weight.

In comparing the results of different authors, the following fact must be borne in mind. A comparison is made between series from countries with entirely different social standards, from towns with widely divergent social structures, from maternity departments with extremely different incidences of premature births and premature mortality, from periods with different birth rates, from war-time and peace-time series. Other differences may also be found.

b. The main source of uncertainty is the following. To what extent is demonstrable mental subnormality a result of combined genetic and social factors, which have led to both a low birth weight and to poor intellectual development evaluated during pre-school or school age?

c. When it is a question of neonatal mortality or of mortality during the first year of life, it is not customary nowadays to publish series of less than 100 cases. When the problem arises of the importance of a low birth weight for the future of the individual, generalized conclusions are often drawn from small series which are subject to large random errors. Moreover, the grounds for the evaluation vary appreciably. Comparisons are not infrequently made between different series of prematurely born children, or between the results obtained for such children and the mean values for schoolchildren of the same age with a normal birth weight. It is not always borne in mind that the respective series may be selected, in that children with an I.Q. below a certain level are not represented in the control series. Moreover, it is unusual to find a report of a control series comparable with regard to the environment and the period of time.

Certain conclusions may obviously be drawn even from relatively small series that are unsatisfactory from certain points of view. However, it is im-

possible to disregard the fact that — one hundred years after the question was first raised in the medical literature — certain essential problems regarding the importance of the low birth weight for the future of the individual have still failed to receive a generally accepted answer. The present investigation is therefore an attempt to approach the problem to some extent on different lines to those previously used.

CHAPTER III

Scope of the Present Investigation

1. The Problem

The following general principles were drawn up for the investigation:

1. The number of prematurely born children in the series should preferably be considerably larger than in earlier investigations.

2. The basic material should be relatively uniform. For this reason, the lowest social stratum should not be included. This was in order to exclude, to some extent, the most markedly unfavourable factors from a genetic and socio-economic point of view.

3. The observation period should be sufficiently long to permit an evaluation of the final social adjustment of those followed up. Previously, the attempts have been confined to an evaluation, some time during childhood, mainly of the motor and mental functions that are only part of the prerequisites for future social adjustment.

4. The follow-up investigation should be based mainly on a search through state and communal records. In these records, the birth weight is not the determining factor and may therefore be considered as an objective evaluation. When going through the records, the examiner should have no advance knowledge of whether the subjects were prematurely born or had a normal birth weight.

5. The comparative material should consist of a comparable control series from the same maternity hospital and from the same period of time, *i.e.*, so-called social twins.

6. The follow-up investigation should, if possible, be made so thoroughly that reliable information could be obtained about every subject in both series. This should be feasible in Sweden, since the country has not been at war for over one hundred years and national registration has been in force for more than two hundred years. This should be particularly easy during and immediately after rationing, as it was necessary to be registered for census purposes in order to obtain ration cards for the most essential foodstuffs. Moreover, infant mortality has always been low in Sweden and, with the exception of the influenza pandemic in 1917 to 1918, we have had no severe epidemic during the present century.

Sweden maintained a strong defence force in 1939 to 1945. All those liable

to conscription, *i.e.*, born between 1902 and 1921 inclusively, were either called up for repeated periods or, if they had earlier been exempted, were re-examined. Thus, the military data with regard to the physical condition and military fitness are particularly complete and up to date.

7. Information concerning those followed up should, if possible, include both positive and negative factors. This would avoid giving — as has most frequently been the case — only the number of extreme negative deviations from the norm.

The points that I have stressed in the aforegoing as being of great advantage in a series obtained in this way obviously imply certain limitations. In the present investigation they were the following.

1. In order to obtain a sufficiently large series and an adequate observation period, the series had to derive from the beginning of this century.

2. In order for the information regarding the birth weight to be reliable, the series had to be assembled from maternity hospitals. During the period in question, 50 to 75 per cent of the parturient women in Stockholm were delivered at a maternity hospital. This involved an over-representation of mothers living in poor housing conditions, unmarried mothers and expected complications during delivery. On the other hand, one of the maternity hospitals in question had a private ward during the whole period and another during part of the period.

3. In order to obtain a more uniform series, it was necessary to confine the investigation to children of mothers registered for census purposes in Stockholm in the year before the birth of the child.

4. The investigation was confined to male subjects. This was because it appeared more likely that information would be found about men than about women in a number of the records to be consulted, *e.g.* the Central Conscript Bureau (*Centrala värnpliktsbyrån*), the Inland Revenue Office (*Skatteverket*), the National Liquor Control Board (*Kontrollstyrelsen*) and the Penal Register (*Straffregistret*).

5. Initially, both children born in wedlock and those born out of wedlock were included, but the investigation was finally confined to the former category. This limitation was made for the following reasons. At the beginning of the 20th century, the children born in wedlock represented a higher social stratum. The number of children born out of wedlock has always been high in Sweden. Between 1901 and 1920, such children comprised 30 per cent of all those born in Stockholm and 13.8 per cent of those born in the whole country. During the same period, they comprised 48.3 per cent of all the prematurely born children discharged alive from the three maternity hospitals in question. The mothers of children born out of wedlock nearly always represented those worst situated in the community. Considerable difficulties

were encountered in attempts to trace them in the same way as the children born in wedlock. The mother not infrequently married another man and the child was known by his surname. In other cases, the children were placed in orphanages or in foster-homes and adoptive homes all over the country. At the beginning of this century, the name of the unmarried mothers was not recorded in the birth records at one of the maternity hospitals in question. Moreover, the mortality was considerably higher among the premature infants born out of wedlock than among those born in wedlock.

In addition, the inclusion of the children born out of wedlock would not have contributed any new feature to my original problem. It would only have made it more difficult to ascertain the importance of the birth weight for the social prognosis. Finally, as mentioned earlier, I did not wish the series to be composed of the children of those worst situated in the community. This would have made it more difficult to evaluate the social variations in a negative direction.

6. Only those prematurely born children discharged alive from the maternity hospital were included. The number of liveborn prematures excluded in this way corresponds approximately to the deaths during the first 14 days of life. I did not consider it worthwhile to make a study of the mortality among prematures during the first weeks of life on the basis of case records 25 to 45 years old. At that time, the data regarding the post-natal state of the child were relatively scanty, and post-mortem examinations were seldom performed on children who died at an early stage.

2. Planning of the Investigation

The two series to be studied were assembled as follows. I went through the birth records at Allmänna Barnbördshuset, Södra Barnbördshuset and Pro Patria from 1902 to 1921 inclusively. These three maternity hospitals were the only ones of importance in Stockholm during the period in question. They will be denoted in the following as ABBH, SBBH and Pro P, respectively. The year 1902 was selected because bound volumes of the records of SBBH existed from this year. I went through altogether 115,980 birth records.

Data concerning the subjects whose development I wished to study were taken consecutively from the records and entered on a separate card for each subject. White cards were used for the basic series and green for the control series; in other respects the cards were identical.

The basic series consisted of all the male children born in wedlock with a birth weight of 2500 grams or less, who had been discharged alive from the

maternity hospital, and whose parents were found in the parish registers of Stockholm in the year preceding their birth.

The control series was assembled as follows. Corresponding to each case in the basic series, I took the boy with the next following case-record number at the same maternity hospital, with a birth weight from 2760 grams up to and including 3750 grams, who had been discharged alive from the hospital, who had been born in wedlock and whose parents were found in the parish registers of Stockholm in the year preceding his birth. These borderline figures for the weight were taken because the mean birth weight for boys was stated to be approximately 3250 grams at the beginning of the century, and I considered it appropriate for the children in the control series to have a birth weight close to the mean.

The following data were entered on the card. — The case-record number at the maternity hospital. — The date of birth. — The name, occupation of the parents and their address. — The majority of children had been christened at the hospital; the christian name of the child could then be obtained from the case record. — Weight and length at birth. — Age of the mother, parity number of the child and the condition of the mother during pregnancy. — All available data regarding the course of delivery and of the child's condition at the hospital. — A calculation (whenever the necessary data were available) of how many weeks before or after calculated term the child was born. — About 20 items were obtained from each record.

The disposition of the birth records differed in all three hospitals and was changed several times during the 20-year period. The data taken from them were therefore checked three times in order as far as possible to exclude faulty transcriptions. Relatively many errors were found in the actual records; this applied in particular to the spelling of the names or the order of the christian names. This led to considerable difficulties in the subsequent identification of the subjects and it was necessary to check with the baptismal registers. The information contained in the baptismal register with regard to the parents is taken from the extract from the parish register that must be presented by the mother at the maternity hospital. Notification to the parish authorities is made according to the entries in the baptismal register. Possible errors are cleared up and corrected in this book.

This check with the baptismal registers disclosed that a number of the unmarried mothers who lived with the child's father had used his surname. In other cases the mother had given a temporary address in Stockholm instead of the address under which she was recorded in the parish register. In the early stages of the investigation, only the subject's name and that of the mother were noted. In many cases this proved inadequate for subsequent identification. In such cases, the baptismal register — but not the hospital

record — provided the name of the father and his date of birth, as well as the exact date on which the family moved into the parish.

When these data had been entered on the cards, they were turned over to the Census Registration Office of the City of Stockholm to be checked with the current address register. This has been in existence since 1926 and lists all those persons who were then or subsequently registered for census purposes in the City of Stockholm. When a subject was found in this list, I obtained his last or present address in Stockholm, his occupation, conscript number, possible change of surname by deed-poll[1] and, when applicable, the date and cause of death. If he had moved from Stockholm after 1926, information was given concerning the date and the address to which he had moved and whether he had once more become resident in Stockholm. In the latter case, his address and date of taking up residence were supplied.

Considerable work was involved in tracing those who were not found in this address register. The register of the parish in which the subject was born was first consulted; inquiries were then made (usually by letter) in all the parishes to which he had subsequently moved. In practically every case, the subject could be traced until January 1st, 1948. If he had died before this date, the date and cause of death were ascertained.

The reason for which some subjects could not be traced, although their disappearance was presumably only apparent, was the following. A number of families had obtained a certificate of altered residence but had failed to move to the place in question. Their plans had presumably changed and they took up residence in a different place. During the first years of the century, it was not encumbent upon the authorities in the parish to which the move had been made to inform the parish in which the person had previously been resident of this move.

Persons who continue to be registered in a parish although they no longer live there, and have failed to obtain a certificate of altered residence, are — after some search has been made — transferred to the parish "list of missing persons." In the majority of cases, this disappearance was presumably only apparent and occurred before national registration reached its present effectivity. I also checked every such case with the "record of missing persons" for the whole country kept by the Central Bureau of Statistics. On practical grounds, I made no further attempts to trace those subjects who had definitely left Sweden.

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[1] During the past 20 years, a considerable number of people in Sweden have changed their surnames by deed-poll. This is in order to avoid confusion, since certain surnames are extremely common. This fact greatly increased the difficulty of tracing the subjects in the present investigation.

It was possible to identify and follow up more than 98 per cent of the subjects in both series.

Both series were then listed in alphabetical order for each maternity hospital separately and the following data noted. Hospital record number. — Complete surname and christian names and, when applicable, change of name by deed-poll. — Date of birth. — Parish in which born. — Last known address. — Conscript number. — The lists were then sent to the various state and communal organs in order to check in their records, which are usually arranged alphabetically, those subjects registered before January 1st, 1948.

The following registers were consulted; the first four cover the whole country and the last four the City of Stockholm only:

1. The Central Conscript Bureau (*Centrala värnpliktsbyrån*).
2. The Penal Register of the National Prison Board (*Kungliga Fångvårdsstyrelsens straffregister*).
3. The Statistical Department and Penal Register of the National Liquor Control Board (*Kungliga Kontrollstyrelsens statistiska avdelning och straffregister*).
4. The Central Register of the National Pensions Board (*Kungliga Pensionsstyrelsens Centralregister*).
5. The Register of Special Classes of the Elementary Schools of the City of Stockholm (*Stockholms stads folkskolors hjälpklassregister*).
6. The Social Welfare Register of the City of Stockholm (*Stockholms stads socialregister*).
7. The Register of the Central Tuberculosis Dispensary of the City of Stockholm (*Stockholms stads centraldispensärsregister*).
8. The Inland Revenue Office of the City of Stockholm (*Stockholms stads skatteverk*).

For the Central Conscript Bureau, a special card had to be filled in for each subject, giving his conscript number and, when possible, his enrolment area. I then obtained information from the various enrolment authorities, based mainly on the military medical sheet. The following data were obtained. Height and weight on enrolment or re-examination. — Number or letter for the fitness classification under which the subject had finally been placed, which may be considered as an evaluation of his bodily constitution. — More severe illnesses (denoted by the military medical code number) during military service or such illness that had prevented him from completing military service. — Military fitness and behaviour. — Possible promotion. — I was also able to obtain additional data concerning the subject's occupation and civil training useful for military purposes.

The Penal Register of the National Prison Board supplied information regarding those subjects who had been convicted, the nature of their offence

and the sentence received. In those cases in which they were found to be without the possession of their senses at the time of the offence (Penal Code § 5:5) or without full possession of their senses (Penal Code § 5:6) this was also stated.

Information was obtained from the Statistical Department and Penal Register of the National Liquor Control Board on such matters as the persons who, since 1936, had been convicted of misdemeanours committed under the influence of alcohol or who had been treated for addiction to alcohol.

The National Pensions Board keeps a list of those persons cared for at its institutions for various diseases, and of those who receive pensions on the grounds of physical or mental disablement and who are incapable of steady employment.

Since 1905, the Elementary Schools of the City of Stockholm have a register of those pupils who attend special classes, and of the time for which they have attended them.

The Social Welfare Register of the City of Stockholm is extremely comprehensive. It contains lists of those persons who have received poor relief, unemployment relief, free hospital care, maternity allowances, children's allowances, institutional care or other financial or social help.

The Tuberculosis Bureau of the City of Stockholm has records dating from the end of the 19th century. Initially, they only included those receiving poor relief, but in recent years they have come to include all known cases notified to the Bureau on the grounds of tuberculosis, suspected tuberculosis and B.C.G. vaccination. Actually, this covers practically every case. Since 1939, acute tuberculosis is a notifiable disease.

Information was obtained from the Inland Revenue Office of the City of Stockholm regarding the "estimated taxable income" for 1947 and 1948. In reality, this is the net income of the subject during 1946 and 1947, the first of these years being tax-free in connexion with the change-over to taxation at the source. The mean figure for these two years is presumably that best in agreement with the actual conditions. Extremely few of the subjects in the series had an unearned income which affected the taxable income; the amount should therefore be an actual expression of the earned income. Information regarding the current occupation was also received from the Inland Revenue Office.

When the information from the respective sources had been assembled, it was transferred to the original cards. The data necessary for the statistical analyses were classified according to a code and transferred to a separate hollerith card for each subject. These were sorted mechanically and the tables thus obtained were submitted to statistical analyses.

3. Statistical Methods

The following statistical methods have been used in this investigation.

The standard deviation σ is calculated with the formula:

$$\sigma = \sqrt{\frac{\sum a^2}{n}}$$

where a is the deviation from the arithmetical mean (M), and n is the number of individuals.

The standard error of the mean, $\varepsilon(M)$ is obtained with the formula:

$$\varepsilon(M) = \frac{\sigma}{\sqrt{n}}$$

where σ is the standard deviation and n the number of individuals.

The standard error of a percentage, $\varepsilon(p)$ is obtained with the formula:

$$\varepsilon(p) = \sqrt{\frac{p(100-p)}{n}}$$

where p is the percentage, and n the number of individuals.

The standard error of a difference, $\varepsilon(D)$ is obtained as follows:

$$\varepsilon(D) = \sqrt{m_1^2 + m_2^2}$$

where m_1 and m_2 are the standard errors of the mean or the percentages on which the calculation of the difference is based.

A difference which is at least three times as great as its standard error is denoted as statistically significant. When the difference amounts to 2 $^1/_2$ to 3 times the standard error, it has, in accordance with Dahlberg's suggestion (1940), been denoted as statistically probable.

In order to obtain a more reliable standard than that afforded by the calculation of the percentages, an estimation of the risk figures according to Dahlberg's method (1939) was made in certain parts of the investigation.

CHAPTER IV

Obstetric and Paediatric Account

1. Cases Excluded

The number of records studied, relating to deliveries at the three maternity hospitals in Stockholm in question during the 20-year period 1902 to 1921 amounted to 115,980. According to these records, 1023 male premature infants, born in wedlock, were discharged alive from hospital during this period (Table 1). In the subsequent course of the investigation, it became necessary to exclude a number of cases from both the series of prematures and the control series. The exclusions were made either because the children did not fulfil the criteria set up or because they could not be traced. A survey is given in Table 2.

In the *series of prematures*, further investigations (particularly in the baptismal registers) showed that 11 children did not fulfil the criteria:

1 was found to be a girl: the sex was given incorrectly in the obstetric record.

3 were not registered for census purposes in Stockholm.

1 had died at the maternity hospital.

1 was not premature: an illegible 3 had been taken for a 2.

5 were born out of wedlock but the mother had stated that she was married.

Thus, with these 11 exclusions, the series consisted of *1012 prematurely born children.*

In the course of the follow-up investigation, the following could not be traced:

3 were stated to be missing by both the parish authorities and the Central Bureau of Statistics.

8 could not be traced: 7 of them had disappeared before 1921 and 1 between 1931 and 1935.

2 had left Sweden before they were 1 year old.

This involved the exclusion of an additional 13 cases. Thus, out of 1012 prematurely born infants, *999, i.e., 98.72 per cent,* could be traced and followed up from birth (1902—1921) until January 1st, 1948.

A similar investigation of the *control series* resulted in the exclusion of the following 5 children:

3 not registered for census purposes in Stockholm.

2 born out of wedlock.

TABLE 1

The original series of prematurely born children discharged alive from the maternity hospitals: distribution in 5-year periods and according to birth in or out of wedlock.

	No. born in wedlock	No. born out of wedlock	Percentage born in wedlock
Total No.	1 023	958	51.6
Years			
1902—1906	192	242	44.3
1907—1911	258	291	47.0
1912—1916	328	239	57.8
1917—1921	245	186	56.9
Maternity hospital			
Pro P	92	19	82.9
ABBH	331	348	48.8
SBBH	600	591	50.4

TABLE 2

Cases excluded from the original series of children born in wedlock.

Maternity hospital	Prematures		Controls	
	Total no.	No. excluded	Total no.	No. excluded
Pro P	92	3	92	3
ABBH	331	10	331	7
SBBH	600	11	600	11
Total	1 023	24	1 023	21

With these 5 exclusions, the control series thus originally consisted of *1018 children.*

In the course of the follow-up investigation, the following could not be traced:

5 were declared missing by both the parish authorities and the Central Bureau of Statistics.

6 could not be traced: 5 of them had disappeared before 1915 and 1 before 1926.

TABLE 3

The final series: distribution according to year of birth and in 5-year periods.

Years	No. of prematures	No. of controls
1902—1906	183	188
1907—1911	253	253
1912—1916	322	321
1917—1921	241	240
Total	999	1 002

5 could not be identified with certainty.

These 16 cases were therefore excluded. Thus, the control series finally consisted of *1002* out of the 1018 children, *i.e., 98.43 per cent;* they could be followed up from birth (1902—1921) until January 31st, 1948.

The distribution of the two series with regard to the number of births during each 5-year period between 1902 and 1921 is shown in Table 3.

2. Multiple Births

In order to compare the series of prematurely born children with those in earlier investigations and with the control series, the classifications described in the following were made.

In the majority of respects, the multiple births have been treated separately. This is because they imply special problems which distinguish them from other premature infants. This procedure has been adopted in only a few of the earlier investigations on the late prognosis for prematurely born children; it appears feasible in the present relatively large series.

According to my calculations, multiple births comprised 17.7 per cent of the original series of male prematures discharged alive from the maternity hospitals in question between 1902 and 1921. The incidence of multiple births among the premature infants born out of wedlock was *11.8 per cent*, the corresponding figure for those born in wedlock being *23.6 per cent*. In ANDERSON and LYONS' (1939) survey of the literature regarding the causes of prematurity, the incidence of multiple births among premature infants was stated to range between 6.3 per cent and 22.9 per cent. Multiple births are a more common occurrence in premature births in wedlock than out of wedlock.

The distribution of multiple births in the present series of premature infants is shown in Table 4.

TABLE 4

Plural-born children among the prematures.

Maternity hospital	No. of twins	Birth order		Pairs of twins
		Twin I	Twin II	
Pro P	23	11	12	4
ABBH.....	81	51	30	17[1]
SBBH.....	136	70	66	25
Total.....	240	132	108	46[1]

[1] Including 1 pair of triplets.

Thus, 146 single twins were included in the series of prematures; 15 of them had a twin brother in the control series and 32 had a twin who died at the maternity hospital. In the remaining cases, the twin was either a sister, or a twin brother with a birth weight outside the range of the present investigation.

In the control series, there were 21 twins; 2 of them had a twin in the same series, 15 had twin brothers in the series of prematures and 2 were single twins.

Four triplets were present in the series; they comprised those surviving out of the 3 triplet births in the series of prematures. There were no triplets in the control series.

3. Birth Weight

The number of premature infants with an extremely low birth weight is relatively small in the present series (Table 5). This could, however, be expected in view of the fact that it consisted of the children discharged alive from a

TABLE 5

Distribution of the children in the different birth weight groups: prematures and controls.

Birth weight: grams	No. of children	Birth weight: grams	No. of children
1 010—1 250.....	5	2 760—3 000	141
1 260—1 500.....	16	3 010—3 250	213
1 510—1 750.....	59	3 260—3 500	306
1 760—2 000.....	116	3 510—3 750	342
2 010—2 250.....	300		
2 260—2 500.....	503		
Total.....	999	Total	1 002

TABLE 6

Distribution of the children according to birth weight and single or multiple birth.[1]

Birth weight: grams	No. of prematures		No. of controls	
	Single-born 1.	Plural-born 2.	Single-born 3.	Plural-born 4.
1 010—2 000	121	75		
2 210—2 250	212	88		
2 260—2 500	426	77		
2 760—3 250			339	15
3 260—3 750			642	6
Total	759	240	981	21

maternity hospital during a period when the mortality among premature infants was extremely high. The series consisted only of children born in wedlock and only certain weight groups were included in the control series. For these reasons, the mean birth weight in the control series exceeded that originally stipulated, *i.e.*, 3 250 grams, which was the figure given as the mean birth weight for male children during the period in question.

In the subsequent treatment of the material, the classification shown in Table 6[1] was that mainly used.

This combination was made in order to obtain larger groups for the statistical analyses.

The percentage of twin births among the premature infants was found to be greater the lower the birth weight group. It is seen from the table that the incidence in rising order of birth weight in the three groups was *38.3 per cent*, *29.3 per cent* and *15.3 per cent*, respectively.

4. Length at Birth

The length at birth was given in almost every case for those children born at ABBH and SBBH, but it was not noted in the records at Pro P. during the period in question. The length at birth and the mean length for single births and multiple births, respectively, in the different weight groups are recorded in Table 7.

[1] In the tables, the following denotations are used for the respective groups:
1 = single-born premature infants
2 = plural-born premature infants
3 = single-born controls
4 = plural-born controls.

TABLE 7

Length at birth in centimeters: prematures and controls.
($M \pm \varepsilon(M)$ = Mean ± standard error of the mean. σ = Standard deviation.)

Birth weight: grams	Single-born children			Plural-born children		
	n	$M \pm \varepsilon(M)$	σ	n	$M \pm \varepsilon(M)$	σ
1 010—2 000	111	42.7 ± 0.21	2.2	71	42.5 ± 0.28	2.4
2 010—2 250	195	45.7 ± 0.13	1.8	75	45.5 ± 0.20	1.8
2 260—2 500	385	46.8 ± 0.08	1.6	70	45.9 ± 0.18	1.5
2 760—3 250	315	49.4 ± 0.09	1.6	14	48.3	
3 260—3 750	578	51.5 ± 0.06	1.5	4	50.3	

5. Degree of Prematurity

The number of days before or after calculated term at which the child was born was calculated in the same way for both series. The calculations were based on von Wachenfeldt's table (1926) and the time stated as the week during which the child was born in relationship to the last menstrual period. Exact data regarding the last menstrual period were, however, lacking in many of the cases. Only those cases were included in these calculations in which a date approximate to within a few days was given and when there was not too great a discrepancy between this date and the possible statement of the first observation of the foetal movements (Table 8).

TABLE 8

Number of days before calculated term at which birth took place: prematures and controls.
($M \pm \varepsilon(M)$ = Mean ± standard error of the mean. σ = Standard deviation.)

Birth weight: grams	Single-born children			Plural-born children		
	n	$M \pm \varepsilon(M)$	σ	n	$M \pm \varepsilon(M)$	σ
1 010—2 000	107	43.3 ± 2.3	24.1	69	36.6 ± 2.2	17.9
2 010—2 250	183	28.5 ± 1.6	22.2	74	30.1 ± 2.4	20.9
2 260—2 500	367	22.8 ± 1.1	20.8	66	24.3 ± 2.4	19.8
2 760—3 250	296	8.1 ± 0.9	15.5		14.1	
3 260—3 750	549	2.2 ± 0.7	15.2		5.8	

6. Maternal Age

In series of premature infants, the maternal age usually differs from the average in that young, unmarried mothers are over-represented. On the other hand, it is often stated that mothers in the higher age groups are found to a greater extent than could be expected. In the present series, the children born out of wedlock were not included. The age of the mothers at the time of the delivery in question is shown in Table 9; a statement of the age was lacking in two cases.

TABLE 9

Mean age of the mothers at delivery: mothers of prematures and of controls. ($M \pm \varepsilon(M)$ = Mean $\pm$ standard error of the mean. σ = Standard deviation.)

Birth weight: grams	Single-born children			Plural-born children		
	n	$M \pm \varepsilon(M)$	σ	n	$M \pm \varepsilon(M)$	σ
1 010—2 000.....	121	29.1 ± 0.5	5.9	75	30.6 ± 0.7	6.4
2 010—2 250.....	212	28.9 ± 0.4	5.9	88	30.0 ± 0.6	5.2
2 260—2 500.....	426	28.7 ± 0.4	5.7	77	30.6 ± 0.6	5.6
2 760—3 250.....	339	28.8 ± 0.3	6.0	15	29.6	
3 260—3 750.....	640	28.7 ± 0.2	5.3	6	28.7	

The difference between the mean age of the mothers in the whole series of single premature births and that in the series of multiple premature births is *1.6 ± 0.43 per cent* and is thus statistically significant. In the series of prematures and the control series, respectively, *59.5 ± 1.8 per cent* and *59.4 ± 1.8 per cent*, respectively, of the mothers of the single-born infants were below 30 years of age, whereas the corresponding figure for the premature multiple births is only *48.8 ± 3.2 per cent*. In the present series, many of the older mothers gave birth to plural-born infants. This is in agreement with the established fact that the incidence of twin births increases with a rise in the maternal age. In the present series, no relationship was found between the mean maternal age and the birth weight of the single-born infants.

According to the Statistical Year Book of Sweden the mean age for the married parturient women in the whole country was 31.52 years in 1901—1910 and 31.23 years in 1911—1920.

7. Earlier Pregnancies

Information could also be obtained from the obstetric records regarding whether any earlier pregnancy had been terminated by spontaneous or induced abortion. The statements may be considered as fairly reliable, since

TABLE 10

Earlier abnormal terminations of pregnancy in multiparae: mothers of prematures and of controls.

Birth weight: grams (current pregnancy)	No. of multiparae	No. of earlier		Percentage of abnormal terminations
		Abortions	Stillbirths	
Single births				
1 010—2 000	72	18	8	36.1
2 010—2 250	131	37	5	32.1
2 260—2 500	243	39	8	19.3
2 760—3 250	189	24	6	15.9
3 260—3 750	433	54	8	14.3
Multiple births				
1 010—2 000	50	6	1	14.0
2 010—2 250	56	7	3	17.7
2 260—2 500	55	5	2	12.7

the patients were married women and therefore had less reason to deny such an incident than unmarried women. The series of married mothers with full-term infants had also been questioned on this matter; in my opinion this fact permits fairly definite conclusions to be drawn. It is, however, possible that more detailed inquiries were made of the women admitted for delivery before expected term than of those in the control series. The information regarding earlier premature births was somewhat uncertain, since no heading for such statements was provided in the records. Moreover, the current norms for prematurity still varied appreciably. I did not, therefore, consider a statistical analysis of these data to be warranted. It may also be mentioned that Clason (1932) demonstrated that certain women exhibit a tendency to give birth consistently to underweight or overweight babies, respectively.

Table 10 shows the incidence of earlier abnormal terminations of pregnancy in the multiparae in the series, in cases of single births and multiple births, respectively. In the latter case, the inclusion in some cases of both twins of one mother must be taken into account as a possible source of error.

Altogether *14.9 per cent* of the mothers of the premature plural-born children had earlier had abortions or stillborn infants, as compared to *14.8 per cent* in the control series as a whole and *25.8 per cent* of the mothers of the premature single-born infants. Moreover, in the last-mentioned category, the number of earlier abnormal terminations of pregnancy appeared to be greater the lower the birth weight of the infant in the current pregnancy.

If, however, the number of earlier pregnancies was used as the norm for the multiparae in both series, the conditions were the following. In the mothers of the premature single-born infants, 115 out of 1130 earlier pregnancies, *i.e., 10.2 ± 0.9 per cent,* had terminated in abortion or stillbirth. The corresponding figure for the mothers of single-born infants in the control series was 92 out of 1389 pregnancies, *i.e., 6.6 ± 0.7 per cent,* and for the mothers of premature plural-born children 24 out of 454, *i.e., 5.3 ± 1.1 per cent.*

The difference between the respective categories in the series of prematurely born infants is *4.9 ± 1.4 per cent* and between the mothers of single-born children in the two series *3.6 ± 1.1 per cent;* thus both differences are statistically significant.

8. Parity Number

The parity number of the current pregnancy also plays some role. It is usually stated either according to obstetric standards as the number of pregnancies started, or according to mainly paediatric standards as the ordinal number of the children actually born. In the following, it is given as the ordinal number of the liveborn children, since abortions and stillbirths were dealt with in the preceding section. The corresponding figure for the number of pregnancies started is given in brackets.

In *44.3 ± 1.8 per cent* (41.2 per cent) of the single births in the series of prematures, the child was the first child. The corresponding figure for the single births in the control series is *37.9 ± 1.5 per cent* (36.3 per cent) and for the multiple births in the series of prematures *33.8 ± 3.0 per cent* (32.9 per cent). According to the official statistics for maternity hospitals in Sweden (*cit.* from Thorén) the incidence of primiparae was 42.1 per cent in 1915 and 45.1 per cent in 1920. The difference between the incidence of first parities in the single births and multiple births in the series of prematurely born children is statistically significant, whereas the difference in this respect between the single births in the two series is probable. The respective figures are *10.5 ± 3.5 per cent* and *6.4 ± 2.3 per cent.*

Of the premature single-born children, *16.2 per cent* had a birth parity among the liveborn children higher than three, the corresponding figure for the single-born children in the control series being *17.5 per cent* and for the premature plural-born children *25.8 per cent.* The difference between the two categories of prematures is *9.6 ± 3.3 per cent* and is thus almost statistically significant. The higher birth-order number of the plural-born children is presumably associated with the established fact that the tendency to multiple births is more pronounced in older mothers.

9. Social Groups

The social group to which the father was assigned was based on the statement of his occupation made by the mother on admission to the maternity hospital. In some cases this was corrected from the notation in the baptismal register, which is taken from the extract from the parish register.

A classification was made into three social groups, according to the standards used in Sweden since 1911 in the official election statistics for the election of members of the Riksdag. To group I belong, for example, higher officials, directors of various kinds, and those with academic degrees or corresponding professional qualifications. Group II comprises such occupations as workshop foremen, office workers, non-commissioned officers or other positions requiring special but not academic qualifications and proprietors of small factories, or craftsmen, and farmers on a moderately large scale. To group III are referred employees in industrial work, trades or agriculture and those with lower positions in occupations requiring little or no specialized training.

TABLE 11

Social group of father.

Social group	No. of prematures 1+2.	No. of controls 3+4.
I	45	45
II	278	259
III	675	695
Unknown	1	3
Total	999	1 002

TABLE 12

Percentage distribution of the children in the respective social groups of the father.

Social group	Percentage of prematures		Percentage of controls	
	Single-born	Plural-born	Single-born	Plural-born
I	4.1	5.8	4.5	5
II	27.8	27.9	26.2	14
III	68.1	66.3	69.3	81
Total	100.0	100.0	100.0	100.0

The main difficulties encountered in such a classification were in the borderline cases and for certain manual workers when the same denotation is used for the proprietor and for his employees. However, in both cases the probabilities of faulty classifications apply equally to both series.

The classification of the present series into the three aforementioned social groups according to the occupation of the father is recorded in Table 11.

The percentage distribution of the social groups with a division of the children into single births and multiple births is shown in Table 12.

10. Health of the Mother during Pregnancy

In all the hospital records, a special heading was devoted to the condition of the mother during the current pregnancy. Information on this matter was, however, lacking in *26.1 per cent* of the premature single-born infants, in *28.3 per cent* of the premature plural-born infants and in *31.5 per cent* of the single-born infants in the control series. The relatively low incidence of missing statements for the mothers of both the premature single-born infants and, to some extent, for those of the premature plural-born infants indicates that a more detailed history was taken in cases of prematurity. The difference between the two categories of mothers with premature infants also leads one to suspect that, in many cases, multiple birth was accepted as the cause of prematurity.

In *35.3 per cent*, *21.7 per cent* and *41.4 per cent*, respectively, of the cases in the aforementioned categories the mother was stated to have been healthy during the current pregnancy. Presumably, inquiries regarding the state of health were always made when taking the history. It is therefore highly probable that any more severe illness would have been recorded. It thus appears possible to assume that when statements concerning the condition during pregnancy were lacking, the mother was healthy. On this basis, the incidence of healthy or presumably healthy mothers was *61.4 per cent* for the premature single-born infants, *50.0 per cent* for the premature plural-born infants and *72.9 per cent* for the single-born controls.

Table 13 shows the distribution according to the diagnosis of those mothers who had suffered from some illness or disability during pregnancy, or who had exhibited symptoms of some such nature on admission to hospital. For practical reasons, several conditions have been combined in the table.

A calculation was made with the exclusion of the mothers with proteinuria of less than 0.1 per cent (determined according to Esbach) and of those with molimina or debility. The figures for true pathological conditions during pregnancy in the three groups (the 21 multiple births in the control series are not included in the table) were then as follows. For the premature single

TABLE 13

State of health of the mother during the current pregnancy.

Diagnosis	No. of mothers of single-born prematures 1.	No. of mothers of plural-born prematures 2.	No. of mothers of single-born controls 3.
Total No.	759	240	981
Debility, severe molimina	15	9	15
Trauma, overwork	2	1	1
Myoma, uterine prolapse	7	1	3
Early haemorrhage	14	2	7
Late haemorrhage	11	1	2
Proteinuria up to 0.1 % (Esbach)	102	50	146
» 0.1—0.2 % »	29	12	23
Eclampsia, pre-eclampsia	37	13	14
Syphilis	3	3	3
Various forms of tuberculosis	12	3	8
Urinary tract infections	12	1	7
Respiratory tract infections	13	2	8
Other more severe infections	9	11	9
Heart disease	15	3	6
Miscellaneous	13	8	14
Total	294	120	266
Percentage	38.7±1.8	50.0±3.2	27.1±1.4
True pathological conditions: No.	177	61	105
Percentage	23.3±1.5	25.4±2.8	10.7±1.0
Difference: 1—3: 12.6±1.8 per cent			

births, 177 out of 759 (*30.6 per cent*); for the premature multiple births 61 out of 240 (*25.4 per cent*), in this category the mothers with two children being recorded twice; and for single births in the control series 105 out of 981 (*10.7 per cent*).

This analysis showed that the conditions were similar to those found in the majority of other series of prematurely born infants. Thus, abruption of the placenta or different degrees of placenta praevia are important causative factors of premature birth, especially in single pregnancies. Moreover, various forms of proteinuria are a frequent finding in the mothers of both single-born and plural-born premature infants. To these common causes of prematurity must be added chronic and acute infections and cardiac diseases in the mother.

11. Pathological Conditions Associated with Labour

The statements in the obstetric records of abnormalities associated with labour are summarized in Table 14. On practical grounds, certain fairly similar conditions have been recorded under the same heading.

When a pelvic deformity was present, this was usually the reason for inducing labour before term. The distribution in the present series is in agreement with the statements in the literature of an increased incidence of placental abnormalities and of atypical presentations in premature births. In the multiple births, however, the presentations noted in the table were due, to some extent, to the termination of labour with podalic version and extraction. In the present series, protracted labour denotes an interval of more than 24 hours between the onset of labour and birth of the child. The cases of intra-uterine foetal asphyxia have not been included here, but have been recorded together with asphyxia occurring post-natally.

TABLE 14

Complications associated with labour.

Complication	No. of prematures		No. of controls
	Single-born 1.	Plural-born 2.	Single-born 3.
Total No.	759	240	981
Pelvic deformity	6	0	1
Hydramnios	2	1	2
Premature rupture of membranes	8	0	2
Precipitate birth	18	1	5
Abruption or abnormal position of placenta	21	2	2
Lesions of placenta	3	0	0
Breech presentation	35	31	15
Foot presentation	17	34	2
Transverse presentation	3	1	1
Brow presentation	1	1	0
Prolonged labour	88	16	129
Umbilical cord round neck	17	2	17
More than one complication	17	4	3
Total	236	93	179
Percentage	31.1 ± 1.7	38.7 ± 3.1	18.2 ± 1.2

Difference: 1—3: 12.9 ± 2.1 per cent
1—2: 7.6 ± 3.5 » »

12. Obstetric Interventions during Labour

The obstetric interventions during labour are recorded in Table 15. In this respect as well, the conditions are similar in the present series to those in other series of prematurely born infants. At the beginning of this century, induced labour before term was a relatively common procedure in, for example, cases of pelvic deformity caused by rickets. Podalic version and extraction was also a fairly common intervention in twin births. Forceps were applied more frequently to a large foetus than to a small one.

TABLE 15

Obstetric interventions during labour.

Intervention	No. of prematures		No. of controls
	Single-born 1.	Plural-born 2.	Single-born 3.
Total No.	759	240	981
Induced labour before term	16	2	1
Podalic version and extraction	16	21	4
Low forceps	9	11	23
Mid-plane forceps	12	6	19
Expression	2	0	4
Caesarean section	0	1	0
Total	55	41	51
Percentage	7.2±0.9	17.1±2.4	5.2±0.7

Difference: 1—3: 2.0±1.1 per cent
1—2: 9.9±2.6 » »

13. Condition of the Infant

Once the child had been born, few notes regarding the condition were made in the records. It is therefore probable that only the more outstanding abnormalities were recorded. Table 16 shows the observations regarding the postnatal state of the child found in the records. Severe asphyxia is not included in this table but is accounted for separately in Table 17.

It must be borne in mind that the infants concerned were those discharged alive from a maternity hospital.

As more severe asphyxia before the end of labour are denoted those cases in which the record noted slow foetal movements for some time, or a high meconium content in the liquor amnii. Also recorded in the table are those cases in which there was a note of a more severe cyanotic attack post-natally. Only those cases are included under the heading of foetal asphyxia in which this was the only symptom.

TABLE 16

Abnormalities in the children at birth.

Abnormality	No. of prematures		No. of controls
	Single-born 1.	Plural-born 2.	Single-born 3.
Total No.	759	240	981
Malformations	11	2	4
Probable birth injuries	10	0	1
Haemorrhagic disease	3	2	3
Sclerema neonatorum	5	3	1
Corneal opacities	2	0	0
Total	31	7	9
Percentage	4.1±0.7	2.9±1.1	0.9±0.3
Difference: 1—3: 3.2±0.8 per cent 1—2: 1.2±1.3 » »			

TABLE 17

Asphyxia during and after birth.

	No. of prematures		No. of controls
	Single-born 1.	Plural-born 2.	Single-born 3.
Total No.	759	240	981
Foetal asphyxia	21	5	24
Neonatal asphyxia	43	15	28
Total	64	20	52
Percentage	8.4±1.0	8.3±1.8	5.3±0.7
Difference: 1—3: 3.1±1.2 per cent			

14. Summary

I have stressed repeatedly in the account of the material that the present series are selected in that they comprise only male children born in wedlock and discharged alive from the maternity hospital. For these reasons, the series are directly comparable neither with earlier reported series of prematurely born children, nor with corresponding normal conditions for Sweden

TABLE 18

Survey of the obstetric and paediatric differences between the single-born prematures (1) and the single-born controls (3) and between the single-born prematures (1) and the plural-born prematures (2), respectively. The controls are used as the norm.

Characteristic	Single-born prematures (1) compared to single-born controls (3)	Single-born prematures (1) compared to plural-born prematures (2)
Length at birth	1 shorter than 3	1 equal to 2
Birth before calc.term	1 earlier than 3	1 equal to 2
Mean age of mother	1 equal to 3	1 younger than 2
Earlier abortion or stillbirth	1 more common than 3	1 more common than 2
First parity	1 more often than 3	1 more often than 2
Higher than 3rd parity	1 equal to 3	1 more seldom than 2
Social group of father	1 equal to 3	1 lower than 2
State of health of mother	1 poorer than 3	1 equal to 2
Complications during labour	1 more common than 3	1 more seldom than 2
Intervention during labour	1 more common than 3	1 more seldom than 2
Abnormality in child	1 more common than 3	1 more common than 2
Asphyxia in child	1 more common than 3	1 equal to 2

during the period in question. The two series are, however, mutually comparable. I have therefore made a comparison in tabular form between these two series (Table 18). The conditions applying to the single-born children in the control series were used as the norm. The middle column in the table comprises a comparison between the control series and the single-born infants in the series of prematures. The last column records a comparison between the single-born premature infants and the plural-born premature infants.

CHAPTER V

Mortality

1. Total and Percentage Mortality and Mortality Risks

An account has already been given of the way in which the subjects in the series were traced, after discharge from the maternity hospital, through the census records and the parish registers. The follow-up investigation covered the events up to January 1st, 1948. It was possible to ascertain the date and, in practically every case, the cause of death of those who had died before this date.

"Congenital debility" was often given as the only cause of death in prematurely born infants who died within the first few months of discharge from hospital. Actually, the death of the majority of such infants who were healthy on leaving hospital was probably caused by infectious diseases, nutritional disturbances or other illnesses. Post-mortem examination was seldom made of such infants.

In the few cases in which the exact cause of death was not stated, the children had died at the beginning of the century in small country parishes. A diagnosis by a physician was not at that time an absolute requirement, but the clergyman of the parish could make a note of the probable cause of death and the certificate was then signed by a physician before forwarding it to the Central Bureau of Statistics.

With regard to children more than a few months old, the diagnosis of the cause of death may be considered as fairly reliable. Whenever possible in the survey in Table 19, only one diagnosis has been recorded, *i. e.*, the illness given in the death certificate as the main cause of death. In the subsequent analyses, these diagnoses were assembled into a number of main groups. It is principally in the case of the main groups comprising convulsive diseases and heart diseases that the earlier conceptions and those of today differ essentially. Under the heading of convulsive diseases were such divergent conditions as spasmophilia, encephalitis, and sequelae of cerebral haemorrhages. When heart disease was given as the cause of death, it was seldom stated whether the disease was congenital or acquired.

It may be considered that little of practical value is gained by a study of the mortality during the first years of life among children born so long ago. During these years, they lived under social and medical conditions that no

TABLE 19

Number of deaths in the series during the whole observation period: distribution according to the cause.

Cause of death	Prematures		Controls	
	Single-born 1.	Plural-born 2.	Single-born 3.	Plural-born 4.
Unknown	1	1	2	—
Congenital debility	49	27	2	—
Various forms of tuberculosis	26	6	39	—
Congenital syphilis	3	—	—	—
Resp. tract infections	45	27	31	—
Gastro-enteritis	17	8	10	—
Pertussis	7	5	3	—
Measles	7	1	1	—
Diphtheria	3	2	6	—
Scarlatina	5	1	7	—
Poliomyelitis	3	—	3	—
Other acute infections	12	5	7	1
Nephritis	3	—	4	—
Convulsive diseases	5	3	5	1
Intracranial haemorrhage, Little's disease	3	1	—	—
Pylorospasm	2	—	1	—
Cong. or acq. heart disease	9	3	9	—
Accidents, murder	12	4	11	—
Suicide	2	—	—	1
Malformations	1	—	1	—
Miscellaneous	6	1	11	1
More than one cause	4	—	1	—
Total	225	94	154	4

longer exist in Sweden, with its far advanced social equality, good provisions for the care of sick children and widespread prophylactic child welfare. As a result, the infant mortality rate in Sweden is the lowest in the world. It would therefore seem appropriate to look forward, rather than to dwell on matters that belong more to the historical aspects of medicine.

It must, however, be recalled that we are dealing with those age groups who are at present engaged in industrial production and who are of utmost importance for the smooth functioning of the community. Those factors which have resulted in the present structure of these age groups cannot be considered as lacking in topical interest. Moreover, the mortality among

TABLE 20

Mortality and percentage mortality from all causes and from acute infectious diseases: prematures and controls.

	Prematures		Controls
	Single-born 1.	Plural-born 2.	Single-born 3.
Total No.	759	240	981
Total mortality	225	94	154
Percentage	29.6±1.7	39.2±3.2	15.7±1.2
Mortality from acute infect. diseases	99	49	68
Percentage	13.0±1.2	20.4±2.6	7.0±0.8

Differences in total mortality: 1—3: 13.9±2.1 per cent
1—2: 9.6±3.6 » »
Differences in mortality from acute infectious diseases: 1—3: 6.0±1.5 » »
1—2: 7.4±2.9 » »

premature infants in Sweden has not declined to the same extent as that among full-term infants. The mortality among prematures is at present responsible for somewhat more than half of the total infant mortality (WALLGREN 1941, GYLLENSWÄRD 1946). Thus, 20—25 per cent of the premature infants in Sweden still die during their first year of life (ALM 1948). It is easier to ascertain the extent to which prematurely born infants are handicapped as compared to full-term infants by a study of those exposed to severe stresses at a time when the therapeutic possibilities were considerably less than at the present day.

Many of the children in my series were born or grew up during World War I which, even in Sweden, implied nutritional privation. They did not have the benefit of prophylaxis against rickets in the modern sense and they were exposed to the influenza pandemic of 1917—1918. Investigations of the mortality among prematurely born children during these years have been made by other workers but, as a rule, the follow-up period was short and the percentage that could be traced was relatively small. Moreover, no comparisons were made with control series comparable from the social and economic point of view.

The total mortality in the respective series in the present investigation up to January 1st, 1948 is shown in Table 19. As already mentioned, this does not include the deaths at the maternity hospitals.

It may be inferred from Table 20 that there is a statistically significant difference between the total mortality among the single-born prematures

TABLE 21

Total mortality: percentage distribution by different causes (prematures and controls).

Cause of death	Prematures		Controls Single-born 3.
	Single-born 1.	Plural-born 2.	
Congenital debility	21.8	28.9	1.3
Various forms of tuberculosis	11.6	6.4	25.3
Resp. tract infections	20.0	28.7	20.1
Gastro-enteritis	7.6	8.5	6.5
Other acute infections	6.7	5.3	4.5
Pertussis, measles	6.2	6.4	2.6
Diphtheria, scarlatina	3.6	3.2	8.4
Convulsive diseases	3.6	4.3	3.2
Heart disease	4.0	3.2	5.8
Miscellaneous	14.9	5.1	22.3
Total	100.0	100.0	100.0

and the single-born controls. The difference in this respect between the two series of prematures is statistically probable. Infectious diseases appear to be responsible for a considerably larger number of deaths among the prematurely born children than among the controls. Moreover, as stated earlier, it is probable that a large proportion of the cases in which congenital debility was stated to be the only cause of death should also be referred to this group. In Table 20, however, only those cases have been included in which an acute infectious disease was stated to be the main cause of death. In this case as well, there is a statistically probable difference between the single-born and the plural-born premature children and a statistically significant difference between the single-born prematures and the single-born controls.

Table 21 shows the distribution of the main causes of death calculated on the total mortality in each series.

A striking feature in all these tables is the high mortality among the premature plural-born children, which is not found in present statistics from the same maternity hospitals. This excess mortality is present mainly in the group of acute infectious diseases.

A relatively large number of the deaths in the control series were caused by tuberculosis. A survey of the incidence and mortality of tuberculosis will be given in a later chapter (pp. 77—79).

The lower the birth weight of prematurely born children, the greater is the mortality risk. The mortality in the various weight groups in the respective

TABLE 22

Percentage mortality in the different birth weight groups: prematures and controls.

Birth weight: grams	Single births		Multiple births	
	No.	Deaths in per cent	No.	Deaths in per cent
1 010—2 000	121	27.3 ± 4.1	75	34.6 ± 5.5
2 010—2 250	212	15.6 ± 2.5	88	23.9 ± 4.5
2 260—2 500	426	13.4 ± 1.7	77	22.1 ± 4.7
2 760—3 250	339	6.8 ± 1.4		
3 260—3 750	642	4.0 ± 0.8		

series is recorded in Table 22, in which only the deaths during the first year of life are taken into account.

Even though the figures in Table 22 refer only to the mortality up to one year of age, the conditions are similar up to five years of age. The mortality rate was found to fall with a rising birth weight and the relative mortality rate among the premature plural-born children was consistently higher, showing no tendency to fall in relationship to that of the premature single-born children. On the other hand, a calculation of the mortality after the age of five years until the end of the observation period gave the following figures: for the 591 premature single-born children *10.1 ± 1.2 per cent*, for the 153 premature plural-born children *5.2 ± 1.8 per cent* and for the 889 single-born controls *7.1 ± 0.9 per cent*. The difference between the premature single-born children and the premature plural-born children is *4.9 ± 2.2 per cent* and between the single-born prematures and the single-born controls *3.0 ± 1.5 per cent*. Thus, the difference is significant in neither case.

It has been stressed repeatedly in the literature that social and economic factors influence infant mortality and that of premature infants in particular. I therefore calculated the mortality rate in the present series with a classification according to the social group to which the father belonged. Only the mortality during the first two years of life was included, since the difference in this respect between the series was still large at this age (Table 23).

It may be inferred from the table that the differences between all three series are statistically significant in social group III; this also applies to the difference between the single-born prematures and the single-born controls in social group II. The mortality was consistently highest among the premature plural-born children.

It has long been an established fact that the mother's state of health during pregnancy greatly influences the condition of her child at birth. Its

TABLE 23

Percentage mortality during the first two years of life: classification according to the social group of the father.

Social group	Prematures				Controls Single-born 3.	
	Single-born 1.		Plural-born 2.			
	No.	Deaths in per cent	No.	Deaths in per cent	No.	Deaths in per cent
I.	31	3.2±3.2	14	7.1±6.9	44	—
II.	211	18.5±2.7	67	26.7±5.4	256	7.0±1.6
III.	516	22.6±1.8	159	40.1±3.9	678	9.4±1.1

Differences: II. 1—3: 11.5±3.1 per cent
II. 1—2: 8.2±6.0 » »
III. 1—3: 13.2±2.2 » »
III. 1—2: 17.5±4.3 » »

effect on neonatal mortality is particularly marked, but this mortality is not included in the present investigation. The effect may, however, be of longer duration. I therefore calculated the mortality rate during the first year of life, after discharge from the maternity hospital, in relationship to the existing information about the state of health of the mother (Table 24)

TABLE 24

Mortality during the first year of life: classification according to the state of health of the mother during pregnancy.

State of health of mother	Prematures				Controls Single-born 3.	
	Single-born 1.		Plural-born 2.			
	No.	Deaths in per cent	No.	Deaths in per cent	No.	Deaths in per cent
Presumably healthy I	465	1.6±0.6	120	3.1±1.6	715	0.5±0.3
Slight ill-health II	117	10.3±2.8	59	15.3±4.7	161	4.3±1.6
Illness III	177	20.9±3.1	61	29.5±5.8	105	8.6±2.7
Differences: I—II.....		8.7±2.9		12.2±5.0		3.8±1.6
I—III.....		19.3±3.1		26.4±6.1		8.1±2.8

— *cf.* Chap. IV, Obstetric and Paediatric Account. In this table as well, the fact that mothers of the plural-born children are included more than once must be taken into consideration as a source of error.

The conditions disclosed by the table are those that could be expected, *i.e.*, the mortality was higher among the children of mothers with more severe

TABLE 25

Percentage mortality during the first year of life: classification according to birth weight (prematures and controls).

Birth weight: grams		Months of life		
		0—2	2—12	0—12
Single births				
1 010—2 250	No.	333	286	333
	Deaths in per cent	14.4±1.9	6.6±1.5	19.8±2.2
2 260—2 500	No.	426	395	426
	Deaths in per cent	7.3±1.3	6.6±1.3	13.4±1.7
2 760—3 250	No.	339	327	339
	Deaths in per cent	3.5±1.0	3.4±1.0	6.8±1.7
3 260—3 750	No.	642	634	642
	Deaths in per cent	1.2±0.4	2.8±0.7	4.0±0.8
Multiple births				
1 010—2 250	No.	163	131	163
	Deaths in per cent	19.6±3.1	11.5±2.8	28.8±3.6
2 260—2 500	No.	77	68	77
	Deaths in per cent	11.7±3.7	11.8±3.9	22.1±4.7

illnesses. The remarkable feature is that the differences were so considerable even after discharge from the maternity hospital. Here again, the mortality was highest among the premature plural-born children. The difference between the mortality among the children of presumably healthy mothers and among those of mothers with some severe illness is statistically significant in both the series of single-born and of plural-born premature infants and almost statistically significant for the single-born children in the control series. The difference between the mortality rate for children of healthy mothers and for those of mothers suffering from slight ill-health is significant in the single-born prematures only.

It is evident from Tables 19—24 that the mortality rate was higher among prematurely born than among full-term infants. The question then arises of when the differences become evened out. In order to obtain information on this matter, I calculated the mortality among the premature single-born, premature plural-born and full-term single-born infants, respectively, during the first year of life (Table 25). I wished to take the birth weight factor into account but also to obtain as large as possible a number in each group. The

TABLE 26

Mortality risks in the respective series.

Age: years	Prematures						Controls Single-born 3.		
	Single-born 1.			Plural-born 2.					
	Years of observation	Deaths		Years of observation	Deaths		Years of observation	Deaths	
		No.	Per cent		No.	Per cent		No.	Per cent
0— 1.....	759	123	16.2	240	64	26.7	981	49	5.0
1— 2.....	636	22	3.5	176	15	8.5	932	18	1.9
2— 3.....	612	10	1.6	161	5	3.1	914	16	1.8
3— 4.....	601	4	0.7	156	1	0.6	897	4	0.4
4— 5.....	595	3	0.5	155	2	1.3	893	4	0.4
0— 5.....	3 203	162	5.1	888	87	9.8	4 617	91	2.0
5—10.....	2 921	19	0.7	751	4	0.5	4 423	12	0.3
10—15.....	2 827	5	0.2	743	1	0.1	4 361	5	0.1
15—20.....	2 790	11	0.4	736	2	0.3	4 310	16	0.4
20—25.....	2 727	9	0.3	730	1	0.1	4 217	13	0.3
25—30.....	2 473	7	0.3	656	—	—	3 834	7	0.2
30—35.....	1 707	6	0.4	441	—	—	2 635	4	0.2
35—40.....	888	2	0.2	204	—	—	1 388	4	0.3
40—45.....	294	1	0.3	77	—	—	461	2	0.4

premature infants were therefore divided into two groups only, *i.e.*, those with a birth weight above and below 2250 grams, respectively.

The greatest difference between the respective groups was found during the first two months of life; during the subsequent 10 months, there were no differences between the weight groups in each series separately. In a comparison between the weight groups in the different series, a difference is, however, apparent.

The mortality risks both for each of the first five years of life and thereafter for the whole observation period in 5-year periods are shown in Table 26.

The mortality risk is highest and the differences between the series greatest during the first two to three years of life. During each 5-year period after five years of age, no difference was found in the mortality in the respective series. If, however, all the deaths after five years of age are added together, a tendency is found to a higher mortality among the premature single-born infants, the lowest figure being found for the premature plural-born infants (see p. 6[illegible]).

The present series derive from a period during which relatively few therapeutic aids were available and large families were common. I therefore tried

TABLE 27

Percentage mortality up to two years of age in children with and without older siblings; prematures and controls.

Birth order no.	Prematures				Controls Single-born 3.	
	Single-born 1.		Plural-born 2.			
	No.	Deaths in per cent	No.	Deaths in per cent	No.	Deaths in per cent
I	336	16.1±2.0	81	29.6±5.1	372	5.9±1.2
II	201	21.4±2.9	67	32.8±5.7	291	6.5±1.4
III	98	21.4±4.1	30	36.7±8.8	146	9.6±2.5
>III	123	29.3±4.1	62	43.5±6.3	172	16.2±2.8
Difference: I—>III		13.2±4.6		13.9±8.1		10.3±1.7

to ascertain whether any difference was present in the mortality rate for the first child and for the children with a higher birth order number. I considered that the principal factor that might be expected to increase the mortality rate for the latter category was the risk of infection by older siblings. The mortality rate for children with and without older siblings, respectively, is shown in Table 27.

The difference in the mortality rate for the first child and for the children with more than three older surviving siblings is statistically significant for the single-born children in the control series. Since there is also a tendency to a rise in the mortality rate with an increasing number of siblings in the series of prematures as well, it is probable that the differences would have been statistically significant in a larger series. It may be mentioned that I had no information regarding the length of time for which older siblings had survived.

I also analyzed the incidence of deaths during the first months of life for those born during the various months and seasons of the year. No facts were forthcoming that permitted any conclusions to be drawn from the present series. I was unable to find any seasonal variations in the mortality rate, although the observation period covered 20 years. I did not, however, take the neonatal mortality into consideration.

A study of the causes of death at different ages yielded little information other than that reported by other workers, or confirmation of long established facts. Only a brief summary is therefore given.

The diagnosis "congenital debility" which, as already mentioned, certainly masked a number of conditions of varying nature, was almost exclusively used for children who died during the first two months of life. The children

who died of tuberculosis during their first year of life were mainly those with a mother suffering from this disease. The peaks in the mortality from tuberculosis that have often been shown to occur during the first years of life, during adolescence and in the twenties could also be discerned in the present series.

There were somewhat more deaths from measles and pertussis among both the premature single-born and plural-born children than among the controls. No such difference was found for the mortality from scarlatina or diphtheria.

An account of the mortality in children in Sweden less than five years of age from 1911 to 1945 has been given by STRÖM (1948). He gave the figures for the mortality from various infectious diseases, both for infants and for children of 1—5 years of age. In children born between 1911 and 1925, pertussis was the most common fatal disease of the infectious diseases of childhood. This applied in particular to the first of life, but the mortality was also high during the subsequent four years of life. Up to 1915, similar conditions, although less marked, were found for measles. During these years, there were many deaths from scarlatina, and still more from diphtheria, particularly from 1—5 years of age.

This difference in the age distribution was also apparent in the present series. Thus, scarcely any deaths were caused by diphtheria and scarlatina during the first year of life, whereas more than half the deaths were accounted for by pertussis and measles. The incidence of deaths from measles and pertussis was higher for the prematurely born infants than for the controls during the first year of life, whereas the figures for the mortality from scarlatina and diphtheria were almost the same at a slightly higher age. This confirms the general view that the resistance of prematurely born children to infections increases with rising age.

In the present series, the incidence of deaths due to other infectious diseases was considerably higher for the prematurely born children, particularly during their first year of life. The difference between them and the controls in this respect was exceedingly small after five years of age.

The only widespread epidemic in Sweden during the present century was the pandemic influenza during the last years of World War I. A study of the mortality in the present series during 1916 to 1920 showed an increase in the deaths from certain infectious diseases during 1917 to 1919, a fact that may be attributed to this epidemic. An increasing number of death certificates then gave such diagnoses as epidemic influenza, capillary bronchitis, pneumonia and acute bronchopneumonia.

As stated earlier, the diagnosis of a respiratory tract infection could be considered as reliable only in children over two months of age. For this reason, I confined the calculation to children above this age. This procedure

presumably resulted in too low a figure for the mortality among the prematures, since the two months during which their susceptibility to infection is greatest were excluded. I did not calculate the number in the series at this stage, nor did I divide the prematures into single-born and plural-born children. The relative size of the series may be calculated on the basis of the fact that there were 880 prematurely born children and 961 controls, these being the numbers of those in the final series that survived the age of two months.

Between 1917 and 1919, 20 prematures (single-born and plural-born collectively) died of the aforementioned illnesses after the age of two months, as compared to only 2 of the controls. There is thus a considerable excess mortality among the prematurely born children if, on the basis of the aforementioned figures, calculations are made of the expected mortality in the other series. This also applies in a calculation of the mortality risks on the basis of the existing relationship between the actual number of deaths from infectious diseases (but not from so-called epidemic diesases of childhood) after the age of two months in the final series. The figures are then 87 for the prematures (single births and multiple births collectively) and 41 for the controls.

2. Discussion

The opinions in the literature are at variance with regard to the mortality risks for single-born and plural-born premature infants, respectively. For example, DUNHAM and MCALENNEY (1936) and PECKHAM (1939) concluded on the basis of their investigations that premature twins have a better chance of survival than single-born premature infants. They based their opinion on the assumption that the former infants are relatively more mature than their birth weight indicates. DUFFIELD *et al.* (1939) stressed, on the contrary, that the mortality rate is higher among plural-born than among single-born prematures. They considered this to depend on the higher incidence of extremely low birth weights in multiple births.

In the present series of prematurely born children, the total mortality risk is, with a high degree of probability, higher for the plural-born than for the single-born children (Table 20). According to Table 22, a larger number of the former category than the latter had a birth weight below 2000 grams, the respective figures being *31.3 per cent* and *15.9 per cent.* The mortality was consistently higher in each weight group for the premature plural-born children, the difference even showing a tendency to increase with a rise in the birth weight. Thus, the difference between the mortality for the plural-born and single-born prematures in the respective weight groups is *7.3 per cent*, *8.3 per cent* and *8.7 per cent.* Moreover, it is evident from Table 23

that the difference between the mortality in the two categories of prematurely born children is statistically significant in social group III, *i.e.*, the mortality is higher for the plural-born prematures.

The fact that the mortality rate in the present series was higher for the premature plural-born children than for the single-born prematures, contrary to the observations in other series of prematures, may be dependent on several factors. (I must once more stress that I am dealing only with the mortality after the infants were discharged from the maternity hospital.) My series is somewhat more uniform than earlier series, in that unmarried, often young mothers of poor social and economic status are not included. The mortality among the children of such mothers is often extremely high and they are more infrequently plural-born. In my original series of all those premature infants who were discharged alive from the maternity hospital, the incidence of multiple births among the children born out of wedlock was 11.8 per cent, as compared to 23.6 per cent among those born in wedlock. As stated earlier, the mothers of plural-born premature children in my series were mainly somewhat older multiparae. The initial social status of the plural-born children was possibly slightly better than that of the single-born children (Table 12). In other respects the series is uniform. The results of the investigation therefore contradict the statement that the chances of survival are greater for the plural-born premature infants than for the single-born prematures in comparable series. Two possible explanations of the difference in the mortality rate are the following. A large number of the single-born premature infants may have died at the maternity hospital. The plural-born children often had a higher birth order number, with a resulting greater risk of infection at home (*cf.* Table 27).

Many series of prematurely born children include all types of prematures with an over-representation of the aforementioned category of young mothers of poor social and economic status, who are usually delivered in a maternity hospital. In such cases, the respective figures for the mortality among single-born and plural-born children may differ essentially from those in the present series.

CHAPTER VI

Registration for Census

In the subsequent study of the prognosis, attempts were made to estimate the chances of the subjects being registered in one of the communal welfare registers of the City of Stockholm. It was therefore of interest to ascertain exactly how many of the subjects were registered for census purposes in Stockholm during each of the years during which these registers have existed or during the years covered by the investigation. Attempts were therefore made to follow the moves of the subjects in detail, a procedure which involved considerable difficulties.

No central census register for Stockholm existed before 1926. To obtain information regarding the moves before this date, it was therefore necessary to consult the different parish registers. For this reason, those who were domiciled in Stockholm in 1926 were not, as a rule, followed backwards in time from this date in order to ascertain the presumably small number who had moved from Stockholm and back to the city before this time. It was usually possible to obtain information about those who had definitely left Stockholm before 1926, by following them from one parish register to the next. It nevertheless proved impossible to carry out my original intentions, but the series were instead classified into the following five main groups:

I. Those who were not known to have moved from Stockholm.

II. Those who had presumably lived in Stockholm for more than two-thirds of the observation period.

III. Those who had lived in some other place than Stockholm for between one-third and two-thirds of the observation period.

IV. Those who had lived in some other place than Stockholm for more than two-thirds of the observation period.

V. Those who had left Sweden.

These groups are recorded in tabular form in Table 28.

Subsequently, the sum of groups I and II was, as a rule, used as the comparative norm for estimating the differences in the series for those listed in the communal welfare registers of Stockholm. Unless otherwise stated, the sum of groups I to IV inclusively was used as a comparative norm for the registers covering the whole of Sweden. The differences between the respective series are, however, presumably due to a random distribution. An analysis of a small part of the material in order to follow the moves in detail showed

TABLE 28

Change of domicile (=move from Stockholm) among those surviving until January 1st, 1948.

Domicile group	Prematures				Controls Single-born 3.	
	Single-born 1.		Plural-born 2.			
	No.	Per cent	No.	Per cent	No.	Per cent
I	297	55.6	78	53.8	460	55.6
II	65	12.2	25	17.2	142	17.2
III	66	32.2	22	29.0	136	27.2
IV	88		19		75	
V	18		1		14	
Total	534	100.0	145	100.0	827	100.0
I+II	362	67.8	103	71.0	602	72.8
I—IV incl.	516	96.6	144	99.3	813	98.3

that, if greater Stockholm is regarded as a geographical and economic unit, no difference between the series is present.

After the first few years of life, little difference was found between the mortality in the series of prematurely born children and in that of the controls. No calculations of the risks were made for the different age groups, with the exception of for those listed in the Penal Register and in the Register of the Liquor Control Board. This was because these registers cover the whole of Sweden and the incidence could therefore be calculated without taking into consideration the moves from Stockholm.

CHAPTER VII

Certain Pathological Conditions

1. Mental Subnormality: Special Classes in Stockholm

It was stated in the general principles for the present investigation that the observation period should be as long as possible, since no study has hitherto been made in which a series of prematurely born children has been followed up until adult age. The conditions during pre-school age in particular and those during school age have been investigated by a number of workers.

I had originally intended to include in the investigation the school-leaving certificate. This nevertheless proved impracticable for the years in question. The number of children finishing their schooling in Stockholm during each of the relevant years amounted to up to 10,000. The school-leaving certificates are filed in the archives but not in alphabetical order. It would have been an exceedingly time-consuming task and an expensive one to obtain the information required. I therefore investigated instead of the incidence of those who were unable to follow the instruction in the ordinary classes.

Special classes have been in existence in Stockholm since 1905. Since they have a central register, it was relatively easy to obtain information about those children who were assigned to such classes. According to RAMER (1946) — who made a follow-up study of special-class pupils in Stockholm, born between 1905 and 1917 — until 1919, children were recommended for transfer to special classes by the teachers and examined by the school doctors. After 1919, psychiatrists were appointed to supervise this transfer and the I.Q. was determined in each case.

Up to 1925, there were no special schools for educable mentally deficient children. It is therefore possible that, before this date, a number of such children attended the special classes. In 1920, so-called B classes were established as an intermediate stage between the ordinary classes and the special classes. According to Ramer, they initially contained a heterogeneous collection of moderately mentally defective children, children with poor sight, word-blind children and those physically unfit. For the sake of uniformity, only those children assigned to the special classes were included in the present calculations.

A factor which affected the transfer during the first years, and probably during the greater part of the period in question, is that only the most

TABLE 29

Subjects attending special classes in the City of Stockholm: prematures and controls.

	Prematures		Controls
	Single-born 1.	Plural-born 2.	Single-born 3.
Incidence calculated on	362	103	602
No. attending special classes	12	6	10
Mean no. of yrs. of attendance.....	5.4	5.5	5.1
Percentage.....	3.3±0.9	5.8±2.3	1.7±0.5
Differences: 1—3: 1.6±1.1 per cent			
1—2: 2.5±1.4 » »			

markedly mentally defective children and those who were a disturbing element were assigned to the special classes. Moreover, there was presumably some geographical selection, since only certain schools had such classes. Children attending other schools would have been less likely to be transferred to special classes since they would then have had a long way to go to school.

The distribution of the children in the present series who attended special classes is shown in Table 29. The incidence was calculated on the surviving children who had lived practically all their life in Stockholm and is therefore presumably slightly too high. The figures for the series of prematurely born children are probably affected to a somewhat greater extent, since the incidence of later moves from Stockholm must have been slightly higher in their case (see Table 28). At the time in question, schooling was compulsory from the year in which a child reached 7 years of age up to and including that in which he was 14 years old. The difference in the incidence of moves from Stockholm was certainly less at that time than at the end of the observation period, *i.e.*, at the turn of the year 1947—1948. All the children ended their schooling according to § 48 in the regulations for elementary schools. This implies that they finished their schooling because they were no longer of compulsory school age, but that they had not attained the skill or the knowledge required of children attending ordinary classes.

Table 29 does not include the ineducable mentally defective children and only some of the educable mentally defective children. The records only list those who attended special classes for some time or who were referred for examination. The differences are apparent, although not *statistically* probable, but it need scarcely be pointed out that this is presumably due to the

relatively small number of cases. The majority of authors who have dealt with prematurely born children of school age have reported an increased incidence of children with a low I.Q.

2. Institutional Care and Disability Pensions

During the investigations made in the parish registers in various parts of the country, in the census records and in the Social Welfare Register in Stockholm, the names were obtained of those who had been, or were still, in mental hospitals, institutions for the mentally deficient or other institutions. Diseases constituting an impediment to marriage are noted in the parish registers; this also applies to the census records. Those who are admitted to institutions and those who are permanently incapable of supporting themselves are listed in the records of the Social Welfare Register of the City of Stockholm and of the National Pensions Board. Since state subsidies are paid, it is in the interest of every commune to apply for pensions in such cases. Presumably, the financial situation of only a few of the subjects was such that their institutional care would not have been recorded in one or other of the registers concerned. The figures for such cases are given in Table 30.

It may be mentioned that a number of borderline cases between imbecility and mental disease are cared for in mental hospitals, but the majority of patients suffer from such conditions that can scarcely be considered, according

TABLE 30

Subjects hospitalized in various institutions: prematures and controls.

Type of institution	Prematures		Controls
	Single-born 1.	Plural-born 2.	Single-born 3.
Incidence calculated on	516	144	813
Mental hospitals.....	9	1	8
Inst. for mental defectives.....	9	1	1
Inst. for cripples	5	—	—
Inst. for blind.....	1	—	—
Other institutions.....	1	1	1
Total.....	25	3	10
Percentage	4.8±0.9	2.1±1.2	1.2±0.4

Differences: 1—3: 3.6±1.0 per cent
1—2: 2.7±1.5 » »

to current conceptions, to be associated with premature birth. If those subjects treated at mental hospitals are excluded and the calculations are confined to those in other institutions, the following incidence figures are found. For single-born prematures *3.1 ± 0.8 per cent*, for plural-born prematures *1.4 ± 1.0 per cent* and for the single-born controls *0.25 ± 0.18 per cent*. Thus, in this respect as well, the difference between the single-born prematures and the single-born controls (*2.85 ± 0.78 per cent*) is statistically significant, whereas the difference between the single-born prematures and the plural-born prematures (*1.7 ± 1.2 per cent*) is not significant. Only the number of subjects in the respective forms of institutions are recorded in Table 30. A more detailed account of those listed in the register of the National Pensions Board, together with the diagnoses, is given in the following section.

All those persons who have been granted pensions according to the Act of 1913 are listed in the register of the National Pensions Board. According to this Act, every Swedish citizen is entitled to a pension upon reaching 67 years of age or if, after the age of 16, he is unable to provide for his maintenance by gainful employment, owing to some form of disablement. The Act has subsequently been amended, but the basic principles are the same. In practice, a reduction of the normal capacity for work by two-thirds qualifies for a disablement pension. The absolute and percentage figures for the subjects in the present series granted disablement pensions are recorded in Table 31.

The same comment may be made to this table as to the preceding one, *i.e.*, it may be presumed that only in a few cases was the financial situation such that the subject did not apply for a disablement pension when his earning capacity was reduced to one-third of the normal. In those cases in which communal relief was necessary, the commune applied for a pension.

The main diseases that are considered to be associated with prematurity are various degrees of mental deficiency, epilepsy and spastic paralysis (Little's disease). It is only with regard to these diseases that a statistically significant difference is found between the single-born premature children and the single-born controls.

Opinions are now in agreement regarding the cause of the more common occurrence of these diseases among prematurely born children. They are due to cerebral accidents during birth, arising on the grounds of the fragility of the capillaries, and possibly to asphyxia. A survey is given in the following of the complications during birth in those children suffering from mental deficiency, spastic paralysis or epilepsy.

In the four such cases in the premature plural-born series and the six in the control series, no mention was made in the birth record of any complica-

TABLE 31

Subjects granted pensions for some form of physical or mental disability: prematures and controls.

Diagnosis	Prematures		Controls
	Single-born 1.	Plural-born 2.	Single-born 3.
Incidence calculated on	516	144	813
Ineducable mental deficiency	8	—	—
Educable mental deficiency	2	2	3
Epilepsy	3	1	2
Little's disease	5	1	1
Mental disease	3	—	2
Psychopathy	3	—	3
Neurasthenia	1	—	4
Tuberculosis	3	—	3
"Rheumatic disease"	—	—	4
Sequelae of poliomyelitis	1	—	2
Asthma	—	—	2
Miscellaneous	5	—	4
Total	34	4	30
Total percentage	6.6±1.1	2.8±1.4	3.7±0.7
No. with first 4 diagnoses	18	4	6
Percentage for first 4 diagnoses	3.5±0.8	2.8±1.4	0.7±0.3

Difference for all diagnoses: 1—3: 2.9±1.3 per cent
1—2: 3.8±1.6 » »

Difference for first 4 diagnoses: 1—3: 2.8±0.9 » »
1—2: 0.7±1.6 » »

tion or obstetric intervention during birth. This also applied to 9 out of the 18 cases in the series of premature single-born children; in the remaining 9 cases, the conditions were as follows:

1. Ineducably mentally defective: Abruption of the placenta; dilatation.
2. do. Placenta praevia; induced labour before term.
3. do. Foetal asphyxia.
4. Educably mentally defective: Protracted labour.
5. Epilepsy: do.
6. do. do.; midplane forceps.

7.	Little's disease:	Foetal asphyxia.
8.	do.	Breech presentation.
9.	do.	Premature rupture of the membranes.

The majority of these complications during labour could give rise to, or be a result of, cerebral injury to the child.

3. Tuberculosis: Morbidity and Mortality

A tuberculosis register has existed for a long time in Stockholm; it developed out of one of the poor relief registers. Long illness of the family provider or of his wife naturally resulted in a need for assistance which was dependent partly on medical factors. The considerations therefore included not only a social and economic investigation, but also a medical investigation and medical control. These reports were incorporated into the tuberculosis register which was drawn up in connexion with the intensification of dispensary service about 1940.

From the beginning of this century until the middle of the nineteen-twenties, the diagnosis of tuberculosis was usually based on physical examinations and sputum tests. On the other hand, these cases were often kept under observation for many years and examinations made of other members of the family. After going through the family case-histories, these diagnoses could be denoted as possessing a fairly high degree of accuracy. They were substantiated by the later history of the other members of the family and the large number of children who became secondarily infected with tuberculosis.

When the use of radiography became increasingly widespread, the diagnosis could be made at an earlier stage and with considerable certainty. Since 1939, tuberculosis that may be presumed to be active is a notifiable disease. During the nineteen-forties, anti-tuberculosis work was particularly intense; the results have also been apparent in a sharply decreased incidence of tuberculosis.

The majority of the subjects in the present investigation underwent mass radiography during their military service. Cases of tuberculosis discovered in this way were reported to the dispensary in the place of residence. In addition, mass radiography has become increasingly common in factories and offices during recent years.

I have assembled in one group those subjects who were listed in the tuberculosis register of the City of Stockholm on the grounds that they had been summoned for examination on one or more occasions, owing to a case of tuberculosis in the family or environment. Those who were listed because

TABLE 32

Morbidity in tuberculosis for subjects resident in Stockholm and mortality from tuberculosis after one year of age in the whole country: prematures and controls.

	Prematures		Controls
	Single-born 1.	Plural-born 2.	Single-born 3.
Incidence calculated on	362	103	602
Mild tuberculosis	34	14	65
Severe tuberculosis	6	1	13
Total.....	40	15	78
Percentage	11.0±1.7	14.6±3.5	13.0±1.4
Incidence calculated on	636	176	932
Deaths from tuberculosis after 1 yr. of age	23	5	35
Percentage	3.6±0.7	2.8±1.2	3.8±0.6

Differences in morbidity: 1—3: 2.0±2.1 per cent
1—2: 3.6±3.9 » »

Differences in mortality: 1—3: 0.2±1.0 » »
1—2: 0.8±1.5 » »

they had earlier, or still had, tuberculosis of such a mild degree that they were kept under observation, but were not admitted to a sanatorium or hospital except for examination were referred to another group. Those who were hospitalized for some time and were given active therapy, even if the disease could be denoted as relatively mild, were assembled in another group together with more severe cases, who survived until January 1st, 1948. Frequency calculations were then made for these three groups, based on the number living during the whole observation period and who had spent their whole life, or the greater part of it, in Stockholm.

Those who, after the age of one year but before January 1st, 1948, died of a tuberculous disease somewhere in Sweden are accounted for separately.

The reason for which the mortality in Table 32 was calculated only for those who survived their first year of life is that tuberculosis in the mother has often been stated to be a common cause of premature birth. This applies even to series from fairly recent years. It was formerly fairly difficult to diagnose tuberculosis after only a short observation period and the attitude

towards the prognosis was somewhat fatalistic. It was therefore not uncommon for prematurely born infants to be sent home to the mother, despite a statement in the hospital record that she was suspected to suffer from tuberculosis, or even definitely suffered from it. I therefore assumed that the risks of contracting tuberculosis or of dying of it during the first year of life would be greatest for the single-born premature infants. If a comparison is made between Table 32 and Table 19, no such tendency seems to exist.

If an attempt is made to estimate the risks of infection in the respective series on the basis of the number of known environmental cases, of which the majority consist of tuberculosis in the family, the following figures are found. For the premature single-born children 59, *i.e., 16.3 per cent*, for the premature plural-born children 18, *i.e., 17.4 per cent* and for the single-born controls 91, *i.e., 15.1 per cent.* The calculations are based on the number surviving on January 1st, 1948.

CHAPTER VIII

Characteristics Recorded in Military Registers

1. Introduction

Conscription in the modern meaning of the term has been in force in Sweden since 1872. In 1901 the length of service was prolonged and since then it has undergone some variations. As a rule, those concerned in the present investigation were liable to military service from their 20th to their 47th year inclusive.

On enrolment, the height, weight and chest measurements are recorded. A routine medical examination is made, including testing of the visual acuity and hearing. Any existing medical certificates are given to the medical officer; it is encumbent on him to take them into due consideration when deciding the fitness for service of the conscript. A conscript in possession of a satisfactory medical certificate stating that he is unfit, on physical or mental grounds, for military service is exempt from personal attendance at the enrolment centre.

The conscripts are referred on enrolment to one of the following fitness classifications, depending on the physical constitution and general state of health:

a) Grades 1, 2, 3 and 4: fit for active service.
b) Grade T: temporarily unfit for active service.
c) Grade O: unfit for active service.

Conscripts with an extremely good physical constitution and qualities suitable for strenuous active service are referred to grade 1. To grade 2 are referred those who, on the basis of their physical constitution, bodily health and mental qualities, are considered fully fit for active service. Grade 3 comprises those who, owing to some defect, disability or illness, are only partially fit for active service. Grade 4 consists of those who, for the same reasons as in grade 4 but to a greater extent, are only of limited use for military purposes. Those in grade T are such cases in which the hindrance to military service is assumed to be of a temporary nature; they are re-examined at the age of 22 or later. Grade O implies that the conscript, owing to disability, chronic disease or permanent physical weakness, is unfit for active service.

All those in grade 4 and many of those in grade 3 are required to perform fatigue duties or clerical work but are exempted from bearing arms. The

medical sheet of those in grades 3, 4, T and O must be provided with the medical reason for the classification, using the military medical code number.

The norms for the classification have varied somewhat during the period in question here. In earlier years they were less strict, as may be inferred from the large number who were exempted from military service, particularly during the early nineteen-thirties. For reasons of national economy, fewer of those in the relevant age groups were called up during these years. On the other hand, most of those who had been exempted from military service at this time were re-examined about 1942 and then underwent military training.

Most of the subjects in the present investigation served their first term of military service between 1923 and 1942 and were, as a rule, called up repeatedly between 1939 and 1945. There are scarcely any other age groups in Sweden about whom so much information is available with regard to the physical condition and military suitability. Since the upper borderline for liability to military service was raised from 45 to 47 years of age about 1940, they could be followed up until a relatively high age.

From my point of view, the military records nevertheless had a drawback, in that they only take the current situation into account and only list those who are still liable to military service. Thus, all those born in 1902 and a large number of those born in 1903 had been taken off the records in 1950 before my investigation had been completed. Moreover, those unfit for service were struck from the records in such a way that it was practically impossible to discover the diagnosis in more than a few cases. It was, however, possible — by means of comparisons between the central military registers and the data in the parish registers — to ascertain with considerable certainty the number of those denoted as unfit for military service. Those who were initially exempted from active service were either called up later or were definitely declared to be unfit.

In many cases it was also impossible to obtain complete information regarding the diagnoses for those in grades 3 and 4; no account is therefore given of them here. During the course of their military service, a number of conscripts were moved to another grade than that under which they were originally classified, owing to subsequent physical or mental illness. The fitness classifications recorded in the following are those applying in 1950—1951.

The account of the follow-up investigation in the military records covers the following points. The length and weight on enrolment, which in almost every case was that at the age of 20 years. — The final fitness classification in 1950. — When applicable, military promotion.

2. Height and Weight on Enrolment. — Rohrer's Index

The most striking feature disclosed by Tables 33 and 34 is the following. There is a statistically significant difference between the mean height and mean body weight of the premature single-born children and of the single-born controls, the figures being lower for the former series.

No earlier study has been made of the length and weight of prematurely born children at adult age. The majority of authors who have made follow-up studies of the length and weight have stated that prematurely born children attain the same levels as the full-term children during their first years at school. Even though my series are more uniform from the socio-economic point of view than those of earlier workers, the length and weight of the prematurely born children was less than that of the controls at 20 years of age. The height at this age may be considered as an extremely reliable gauge of the final height.

It was impossible in most cases to obtain information regarding the weight and height of those exempted from military service; they are therefore not included in the aforegoing tables. On the other hand, it would be unreasonable to suppose that they would have been taller and heavier than those who were conscripted. It may be presumed that they were shorter and slighter.

TABLE 33

Mean height on enrolment in military service: classification according to birth weight group (prematures and controls).
($M \pm \varepsilon(M)$ = Mean ± standard error of the mean. σ = Standard deviation.)

Birth weight: grams	Mean height on enrolment: cm					
	Single births			Multiple births		
	n	$M \pm \varepsilon(M)$	σ	n	$M \pm \varepsilon(M)$	σ
1 010—2 000.....	44	170.5±1.3	8.5	28	169.3±1.3	6.8
2 010—2 250.....	117	172.9±0.6	6.4	44	174.6±1.2	7.6
2 260—2 500.....	246	173.2±0.4	6.9	43	173.1±1.0	6.3
2 760—3 250.....	219	174.3±0.4	6.4	10	172.5	
3 260—3 750.....	426	176.3±0.3	6.2	6	175.3	

Mean height for 407 single-born prematures 1. 172.8±0.3 cm
Mean height for 115 plural-born prematures 2. 172.9±0.7 »
Mean height for 645 single-born controls 3. 175.6±0.2 »

Difference: 1—3: 2.8±0.4 cm

TABLE 34

Mean body weight on enrolment in military service: classification according to birth weight group (prematures and controls).
($M \pm \varepsilon(M)$ = Mean ± standard error of the mean. σ = Standard deviation.)

Birth weight: grams	Mean weight on enrolment: kg					
	Single births			Multiple births		
	n	$M \pm \varepsilon(M)$	σ	n	$M \pm \varepsilon(M)$	σ
1 010—2 000.....	35	60.6 ± 1.2	7.2	25	63.2 ± 1.8	9.2
2 010—2 250.....	99	64.0 ± 0.7	7.3	37	67.7 ± 1.1	6.5
2 260—2 500.....	206	64.7 ± 0.6	8.2	35	63.3 ± 1.3	7.7
2 760—3 250.....	183	66.1 ± 0.5	7.3	9	62.8	
3 260—3 750.....	357	67.4 ± 0.4	7.2	2	63.0	

Mean weight for 340 single-born prematures 1. 64.0 ± 0.4 kg
Mean weight for 97 plural-born prematures 2. 65.0 ± 0.8 »
Mean weight for 540 single-born controls 3. 67.0 ± 0.3 »

Difference: 1—3: 3.0 ± 0.5 kg

Conscripts less than 157 centimeters tall are automatically down-graded to grade 3 or 4 and they cannot join the standing army. No upper borderline is fixed, except that conscripts above a certain height are not assigned to some branches of the armed forces.

The mean height of conscripts in Sweden has risen successively during recent years. Thus it increased from 172.1 centimeters in 1921—1925 to 174.5 centimeters in the beginning of the nineteen-forties. This fact has usually been ascribed to the improvement in the nutritional standard and in the general social standard.

On the other hand, prematurely born children have a higher vitamin requirement and a greater need of adequate nutrition, owing to their relatively more rapid growth during the first year of life. The prematurely born children did not have the benefit of prophylaxis against rickets in the modern sense and it is probable that in many cases the controls also suffered from vitamin D deficiency. Moreover, the northerly position of Sweden gives rise to an increased incidence of rickets during the long, dark season. A high incidence of rickets, often of a severe degree, was presumably present in both series, as has been reported in most of the literature from the same period. Deficiency of vitamin D and of other vitamins may have affected the more rapidly growing organism to a greater extent than that growing normally.

Other nutritional, social and economic factors presumably affected both series to the same degree, or may have had a greater effect on the children in the control series. In the latter case, this would be associated with the fact that only those who survived the age of 20 were included in the calculations of the height and body weight. Thus, there was a relatively larger number in the control series with a father belonging to social group III than in the series of prematurely born children.

A comparison was made between those in each series born between 1902 and 1911 and between 1912 and 1921, respectively. It gave the following results. In the single-born prematures, the mean height increased from *171.4 ± 0.6 centimeters* in the first period to *173.6 ± 0.2 centimeters* in the second period. The difference, *2.2 ± 0.8 centimeters*, is statistically probable. The mean height of the plural-born prematures increased correspondingly from *171.2 ± 1.1 centimeters* to *174.2 ± 0.9 centimeters*. The difference is *3.0 ± 1.4 centimeters*. The mean height of the single-born controls increased from *174.8 ± 0.5 centimeters* to *176.1 ± 0.3 centimeters*. The difference is *1.3 ± 0.6 centimeters*. The mean weight in the respective series also increased from the first period to the second, but only to an extent corresponding to the increase in the height.

If any conclusion can be drawn from the aforegoing figures, it is chiefly that there was a more marked tendency to an increase in height among those prematurely born than among the controls. It is therefore possible that the statistically probable difference found in the height might become evened out with improved prophylactic measures.

TABLE 35

Rohrer's index on enrolment in military service.
($M \pm \varepsilon(M)$ = Mean ± standard error of the mean.)

Birth weight: grams	Single births		Multiple births	
	No.	$M \pm \varepsilon(M)$	No.	$M \pm \varepsilon(M)$
1 010—2 000	34	1.23 ± 0.031	25	1.30 ± 0.042
2 010—2 250	98	1.25 ± 0.017	37	1.26 ± 0.025
2 260—2 500	205	1.24 ± 0.012	34	1.20 ± 0.029
2 760—3 250	183	1.25 ± 0.012	9	1.23
3 260—3 750	355	1.23 ± 0.008	2	1.12

A calculation of the body type according to Rohrer's index is recorded in Table 35. No convincing differences whatsoever could be shown between the respective series, nor between the different birth weight groups.

The present series were uniform with regard to the social and economic conditions during the period in question. I therefore consider it to be established that the height and body weight of prematurely born children are lower, even at adult age, than the corresponding figures for full-term children in a comparable control series.

In recent years, prophylaxis against rickets has been increasingly intensified, particularly for prematurely born children. It is impossible to make any statement regarding the extent to which this fact may even out the difference.

3. Fitness Classification

Table 36 shows the fitness classification of the subjects in the present series on enrolment or during the course of their military service. The grade recorded is that noted on the military medical sheet in 1950.

The following comment may be made to Table 36. It includes all those exempted from military service during the 20 years in question, but only those in the other grades born after 1903. If 10 per cent is deducted from grade 0 and the ratios calculated on this total instead, the figures in the table are not affected.

There is thus a statistically probable difference between the single-born prematures and the single-born controls with regard to fulfilling military service, the incidence being higher for the latter series. Presumably, an

TABLE 36

Fitness classification for military service.

Fitness classification	Prematures				Controls Single-born 3.	
	Single-born 1.		Plural-born 2.			
	No.	Per cent	No.	Per cent	No.	Per cent
I	197	39.5 | 56.2	55	42.0 | 62.6	359	48.4 | 63.8
II	83	16.7 |	27	20.6 |	114	15.4 |
III	56	11.2	19	14.5	99	13.4
IV	77	15.5	10	7.6	76	10.3
0	85	17.1	20	15.3	93	12.5
Total	498	100.0	131	100.0	741	100.0
Unknown class	51	9.3	15	10.3	109	12.8

Differences (I + II): 1—3: 7.6 ± 2.8 per cent
» » 1—2: 6.4 ± 4.8 » »

important reason for this difference is the difference in the height and body weight. Because only relatively incomplete data could be obtained concerning the medical reasons for which the conscripts in question were exempted from military service, no attempt has been made to analyze these conditions.

4. Promotion

The question arises of how the conscripts conducted themselves during their military service. I therefore calculated the incidence of those who, on the grounds of military suitability, special training or other factors of value for military purposes, were promoted to warrant officers, non-commissioned officers or officers. Such possibilities were open almost exclusively to those in grades 1 and 2. I therefore calculated the incidence only on the number of those in these two grades. Of the premature single-born children, 42 out of 280, *i.e., 15.0 ± 2.1 per cent*, were promoted. The corresponding figure for the plural-born prematures was only 4 out of 82, *i.e., 4.9 ± 2.4 per cent*, whereas it was 58 out of 473, *i.e., 12.3 ± 1.5 per cent*, for the single-born controls. Strangely enough, there is a statistically significant difference only between the single-born and the plural-born prematures and not between the single-born children in the two series. The differences are *10.1 ± 3.1 per cent* and *2.7 ± 2.6 per cent*, respectively. In any event, the number of premature plural-born children in this analysis is too small to permit any generally applicable conclusions. It is, however, evident that those of the prematurely born children who were called up served their term of military service as satisfactorily as did those in the control series.

CHAPTER IX

Social and Economic Conditions at Adult Age

1. Social Groups

The next step in the investigation was an analysis of the social conditions and social adaptation of the subjects in the series. The occupation, classified into one of the three social groups described earlier (*cf.* p 51) was used as the standard for the evaluation. A comparison was made both between the subjects (sons) and their fathers and between the subjects in the respective series.

There was a difference of 20 years between the oldest and the youngest subjects in my series; the social and economic problems with which they were faced at the same age were not, therefore, the same. In many respects, the social structure of Sweden has undergone radical changes during these 20 years and during the subsequent 26 years up to the end of the investigation period on January 1st, 1948.

Some idea of the background must be given. Although Sweden did not take part in World Wars I and II, the neighbouring countries were involved. As a result, the country was in a state of high military preparedness and was, to a great extent, isolated from the foreign market. The world-wide fluctuations in the state of the market were also reflected in Sweden. There were periods of widespread unemployment, particularly in 1921—1922 and 1932—1934. During the years covered by the investigation, Sweden has become strongly industrialized with a resulting relatively large exodus of the rural population to towns and urban districts.

Central administration has become more extensive, with a resulting larger number of posts in government and communal offices. A considerable increase has also taken place in the number of administrative staff in large private enterprises. Moreover, greater possibilities have been opened up for vocational training and higher studies, even for those with low incomes.

These factors have brought about changes in the social structure and, particularly in Stockholm, a relative increase in the numbers in social groups I and II. For the country as a whole, the respective incidences for social groups I, II and III have, however, remained almost constantly at 5 per cent, 40 per cent and 55 per cent. These figures are based on the election statistics for those entitled to vote for the election of members of the second chamber

TABLE 37

Social group of the surviving subjects in comparison to that of the father (rise or fall in social scale).

Social group compared to that of father	Prematures		Controls
	Single-born 1.	Plural-born 2.	Single-born 3.
Incidence calculated on	544	148	848
Percentage with social group higher than father I	31.6±2.0	19.6±3.3	36.7±1.7
Percentage with social group lower than father II.....	8.5±1,2	9.5±2.4	8.3±0.9
Difference for I: 1—3: 5.1±2.6 per cent			
1—2: 12.0±3.8 » »			
Difference for II: 1—3: 0.2±1.5 » »			
1—2: 1.0±2.1 » »			

of the Riksdag, according to the Act of 1918, *i.e.*, the definite establishment of general franchise for both sexes.

Certain difficulties are encountered in determining the social group to which the individual should be assigned, even though such a classification has been used in the election statistics in Sweden since 1911. In addition to the purely technical difficulties in assessing the occupations, there is the problem caused by the tendency in recent years to up-grade the posts. (For example, some years ago a clerk in an office would have been designated as such, whereas today an individual in the same position is more likely to be called a manager's assistant or a cashier.) This tendency nevertheless has a slighter effect on a classification into three groups only than on a more detailed classification into five social groups.

Another factor of importance is that the social group of the fathers was recorded at the time the child was born, whereas that of the sons was, in most cases, evaluated up to January 1st, 1948. This implies that the social group of the fathers was evaluated at a mean age of about 30 years and that of the sons at the mean age of about 35 years. If it is assumed that the final social standing is usually reached only after 30 years of age, the assessment of the final social position is more definite for the sons than for the fathers. The social group to which the sons belonged could be checked in several registers, whereas that of the fathers was based on the statement in the baptismal register alone. None of these factors should, however, affect a mutual comparison between the series.

TABLE 38

Percentage distribution of the social groups: surviving subjects in the present series and their fathers.

Social group		Prematures		Controls
		Single-born 1.	Plural-born 2.	Single-born 3.
Incidence calculated on (fathers and sons)		544	148	848
Sons	I	10.1 ± 1.3	10.8 ± 2.6	12.4 ± 1.1
	II	44.3 ± 2.1	39.2 ± 4.0	44.0 ± 1.7
	III	45.6 ± 2.1	50.0 ± 4.1	43.6 ± 1.7
Fathers	I	5.5	8.8	4.8
	II	29.0	32.4	26.7
	III	65.4	58.8	68.5

Differences between sons: Social group I: 1—3: 2.3 ± 1.7 per cent
Social group III: 1—3: 2.0 ± 2.7 » »
III: 1—2: 4.4 ± 4.6 » »

Table 37 shows the number of sons in the respective series who rose one or two social groups above that of the father and the number of those who were assigned to one or two groups lower.

In studying Table 37 it must be recalled that the mortality rate was not the same in the respective series. A larger number of the premature plural-born children than of the premature single-born died at an early age and there was a higher mortality among the prematurely born children than among the controls. Moreover, the mortality was consistently highest in social group III and lowest in social group I. From this point of view, the most favourable initial social situation of the surviving sons was in the following order: premature plural-born, premature single-born and controls. On the other hand, the possibilities of rising socially are not so great with a higher initial social position, at any rate when a classification into only three social groups is used. This may explain to some extent the higher incidence of a rise among the single-born than among the plural-born prematures and the tendency to a corresponding difference between the single-born prematures and the single-born controls. No difference whatsoever is present between the respective series as far as a fall in the social scale is concerned. Table 38 shows the percentage distribution among the three social groups for the respective series. No significant differences are present.

To sum up, the following statements may be made. In the present series, the distribution among the three social groups of those of the prematurely born children who survived until adult age was, on the whole, the same as that of the controls. In an attempt to assess the changes occurring in the social status, a difference was found with respect to the number of those who had attained a higher social group than that of the father, the incidence being higher among the single-born controls than among the single-born prematures and among the single-born prematures than among the plural-born prematures. These differences may be partly explained by the fact that the incidence of the surviving subjects belonging initially to the two higher social groups in the respective series was as follows: plural-born prematures *41.2 per cent*, single-born prematures *34.5 per cent*, single-born controls *31.5 per cent.*

2. Public Assistance

The next stage in the investigation of the social and economic prognosis was a study of the Social Welfare Register of the City of Stockholm. I investigated the number of those in the three series who had been recipients of some form of public relief. This applied only to the time during which they were resident in Stockholm, since such relief is usually paid out by the communal authorities.

The various forms of relief were divided into the following four main groups:

a) Poor relief.

b) Unemployment relief.

c) Sickness relief, which was mainly in the form of free hospital care for lengthy illness of the subject himself or, when he was the family provider, of members of his family.

d) Other forms of relief for the subject himself or for members of his family.

The norms for the payment of relief have varied somewhat during the period in question. In general, the possibilities of public assistance have increased considerably during the last 10 years in question, *i.e.*, up to January 1st, 1948. Unemployment relief has also varied; between 1932 and 1934 unemployment was fairly common in Sweden and then affected, out of all those belonging to the various trades unions, 25—30 per cent in the winter and 15—20 per cent in the summer. Normally, there is always some such unemployment, owing to the seasonal nature of certain work. During a boom, it usually affects 5—8 per cent of all workers during the winter and 2—4 per cent during the summer (DAHLBERG and TINGSTEN 1937).

It was not possible to ascertain the exact number of residents in Stockholm, either for each calendar year or for each year of life. No calculations could therefore be made of the chances of receiving some form of relief. Thus,

TABLE 39

Recipients of public relief listed in the Social Welfare Register of the City of Stockholm.

Form of relief	Prematures		Controls Single-born 3.
	Single-born 1.	Plural-born 2.	
Incidence calculated on	362	103	602
Poor relief	20.4 ± 2.1	22.3 ± 4.1	21.8 ± 1.4
Unemployment relief.....	16.9 ± 2.0	21.3 ± 4.0	14.6 ± 1.4
Free hospital care	10.2 ± 1.6	10.7 ± 3.1	12.1 ± 1.3
Other relief	17.1 ± 2.0	23.3 ± 4.2	18.1 ± 1.6
The differences between groups 1 and 3 and 1 and 2, respectively, are not statistically significant			

only the incidence figures are given for the recipients of the various forms of relief (Table 39).

The incidence was calculated on the number of those in residence groups I and II described earlier (*cf.* p 70), *i.e.*, those who had lived for their whole life, or for the greater part of it, in Stockholm. Presumably, this basis for the calculations implies that the figures are somewhat too high. Comparisons can therefore be made only between the respective series. If this raised incidence were to affect one of the series more than the others, it would be the single-born prematures, since the incidence of moves from Stockholm was slightly higher in their case. The controls are presumably those least affected by these raised figures (Table 28).

As a rule, a person may not be the recipient of both poor relief and unemployment relief simultaneously. The number of months during which relief was received was therefore calculated and a comparison made between the number of such months per recipient in the respective series. Finally, the corresponding figures are recorded for those resident in Stockholm for their whole life or the major part of it (Table 40). In this table as well, the figures are certainly too high, since in some cases relief was only received during part of a month. The figures for the respective series are, however, comparable.

Briefly, it may be stated that no significant differences are present between the respective series with regard to the forms of relief in question. The incidence figures were lowest for the premature single-born children in every instance with the exception of unemployment relief. The relatively high incidence of unemployment relief among the premature plural-born children is compensated for by the fact that the number of months during which

TABLE 40

Number of months during which either poor relief or unemployment relief was received.

	Prematures		Controls
	Single-born 1.	Plural born 2.	Single-born 3.
Months of poor relief	1 093	347	1 902
No. of recipients	74	23	131
Months of relief per individual	14.8	15.1	14.5
Months of unemployment relief	591	172	816
No. of recipients	61	22	88
Months of relief per individual	9.7	7.8	9.3
Total months of relief	1 684	519	2 718
No. of residents in Stockholm	362	103	602
No. of months of relief per individual in the series	4.7	5.0	4.5

such relief was received is less than in the other series. As already mentioned, the calculations of the incidence based only on residence groups I and II presumably result in slightly too high percentage figures for the premature single-born children. This also applies, although to a lesser extent, to the premature plural-born children. On the other hand, the surviving children in the series of prematures had a higher initial social situation; this might have given them an advantage over the controls. It is scarcely possible to calculate the numerical importance of these factors. It is, however, evident that the prematurely born children in my series were not a greater burden on the community at adult age than were the controls.

3. Taxable Income

It was shown in the preceding section that no difference existed between the prematurely born children and the controls with respect to the reception of some form of relief from the City of Stockholm. The question then arises of the income derived from employment.

An investigation was therefore made of the taxable income for 1946 and 1947 of those resident in Stockholm. The arithmetic mean for the "estimated taxable income" as assessed by the Inland Revenue Office was used as the norm for the comparison. These years were chosen for the following reasons. The same record number on the income-tax demand was used for both years. The first of these years was tax-free in connexion with the change-over to

TABLE 41

Classification according to net income during 1946 and 1947: subjects resident in Stockholm.

Income: Swedish Crowns	Prematures				Controls Single-born 3.	
	Single-born 1.		Plural-born 2.			
	No.	Per cent	No.	Per cent	No.	Per cent
Less than 4 000	35	9.6	3	3.5	37	6.3
4 000—6 000	150	41.0	44	51.8	205	35.1
6 000—8 000	113	30.9	27	31.8	209	35.8
More than 8 000	68	18.6	11	12.3	133	22.8
Total	366	100.0	85	100.0	584	100.0
Mean	6 540 Sw. Cr		6 010 Sw. Cr		7 190 Sw. Cr	
Median	6 080 Sw. Cr		5 900 Sw. Cr		6 270 Sw. Cr	

taxation at the source. There was a trade boom and practically all available labour was employed.

Estimated taxable income denotes, on broad lines, the taxable income when approved deductions for certain purposes have been made, *i.e.*, it is the net income from employment. It was, as a rule, possible to include only the figures for earned income and not those for unearned income. Information regarding the taxable income for the years in question could be obtained for 366 of the premature single-born children, 85 of the premature plural-born and 584 of the controls. The relevant figures are recorded in Table 41.

A comparison between the median for the respective series shows no definite difference between them. In this respect, the median gives a more accurate estimation than the mean, since a few persons with a particularly high income may influence the figures, owing to a skew distribution.

4. Criminality

In order to obtain further information about the social adaptation of the subjects in my series, I ascertained the number who had been convicted of some crime. This was done by a study of the Penal Register of the National Prison Board, which covers the whole of Sweden. All those over 15 years of age who have been sentenced to imprisonment or to penal servitude since 1900 are listed in this register. It also lists those given a suspended sentence and those released on parole, as well as those whose sentences were remitted on

TABLE 42

Subjects convicted of crimes and listed in the Penal Register.

Nature of crime	Prematures		Controls
	Single-born 1.	Plural-born 2.	Single-born 3.
Incidence calculated on	516	144	813
Crimes against property, receiving stolen goods	17	2	39
Assault and battery	1	—	3
Sexual crimes	3	—	2
Drunken driving	1	—	2
Military defections	2	1	3
Rioting	1	1	1
Miscellaneous	5	1	19
No. convicted	30	5	69
Percentage	5.8 ± 1.0	3.5 ± 1.5	8.5 ± 1.0
No. convicted once	19	2	48
Percentage	3.7	1.4	5.9
Differences: 1—3: 2.7 ± 1.4 per cent 1—2: 2.3 ± 1.9 » »			

the grounds that they were not responsible for their actions at the time of the offence.

The Swedish penal system has been reformed several times during the period in question here and a considerable "humanization" has taken place. Increasing emphasis has been laid on preventive measures and there is a growing trend to give suspended sentences to young persons and to first offenders. There is also an increasing tendency to place the supervision of such persons in the hands of the social welfare authorities, who must be considered to have the greatest possibilities for helping to rehabilitate them.

These reforms, which have mainly influenced the application of the penal laws should not, however, affect comparisons between the present series. The account given here covers the following points. The nature of the crimes, with a classification into a number of main groups. — The incidence of crimes, both for first offenders and for those convicted more than once. — The annual risk and the cumulative risk, calculated for five-year periods. (See Tables 42 and 43.)

A study of these tables discloses the following. There is a tendency to a lower incidence of crimes among those prematurely born than among the

TABLE 43

Percentage annual risk in 5-year periods of being convicted for the first time of crime.

Age: years	No. of yrs. of observation	No. of subjects convicted	Average annual risk: per cent	Cumulative risk: per cent
		Single-born prematures		
15—20	2 770	11	0.40	2.00
20—25	2 667	9	0.34	3.70
25—30	2 405	5	0.21	4.75
30—35	1 663	4	0.24	5.95
35—40	853	1	0.10	6.45
40—45	274			6.95
		Single-born controls		
15—20	4 271	26	0.61	3.05
20—25	4 057	19	0.47	5.40
25—30	3 679	16	0.43	7.55
30—35	2 578	6	0.23	8.70
35—40	1 365	2	0.11	9.25
40—45	484			9.80

controls. A possible contributory factor is that mentioned earlier, *i.e.*, owing to the excess mortality among the prematurely born children in social group III, the initial social status of those children who survived was higher, evaluated according to the social group of the father, than that of the controls.

Premature birth may also have an effect on the personality development. On one hand there is the "rigid type of post-natal care". On the other hand, there must be a particularly strong emotional bond between the mother and these more than normally helpless children. This should apply especially in a series such as the present one. The premature children were all born in wedlock and there was probably a high incidence of those whose birth was welcomed by the parents. In Sweden at any rate, the prematurely born children of unmarried mothers were presumably only infrequently welcome. At the beginning of the present century, it was certainly not the rule for the parties in question to marry only because the woman was pregnant. Owing to the nature of the present investigation, little information was, however, obtained regarding the home conditions and such factors as divorce and broken homes.

5. Temperance Infractions

The Swedish legislation governing the manufacture and sale of alcoholic drinks and the treatment of the abuse of alcohol is somewhat complicated. I shall not, therefore, enter into the details here.

In principle, the supervision of alcohol addicts is in the hands of the communal authorities, the temperance committees (*nykterhetsnämnder*). The control of the sale of alcoholic drinks and of temperance infractions is in the hands of a state organization, the National Liquor Control Board. Since 1932 it has its own penal register, which lists temperance infractions, and crimes and misdemeanours committed under the influence of alcohol. Especially since 1938, the courts are obliged to report to this Board those who have been convicted of such offences.

The account of these matters in the present series (Tables 44 and 45) is recorded, on the whole, in the same way as in the case of other crimes (see preceding section).

This part of the investigation did not show any significant difference between the three series. There is not even a numerical difference between the single-born prematures and the single-born controls. There is a slight tendency, but only at a higher age, to a higher incidence of temperance infractions among those born prematurely. It does not, however, permit any general conclusions to be drawn.

The study of the Penal Register and of the register of the National Liquor Control Board thus showed that, in these respects, those prematurely born

TABLE 44

Incidence of convictions for drunkenness and annual risk of being convicted for the first time of drunkenness.

	Prematures		Controls
	Single-born 1.	Plural-born 2.	Single-born 3.
Incidence calculated on	516	144	813
No. with known convictions	57	22	89
Percentage	11.0 ± 1.4	15.3 ± 3.0	10.9 ± 1.1
No. with one conviction	37	14	59
Percentage	7.2	9.7	7.3
Differences: 1—3: 0.1 ± 1.8 per cent			
1—2: 4.3 ± 3.3 » »			

TABLE 45

Percentage annual risk in 5-year periods of being convicted for the first time of drunkenness.

Age: years	No. of yrs. of observation	No. of subjects convicted	Average annual risk: per cent	Cumulative risk: per cent
		Single-born prematures 1		
15—20	2 788	2	0.07	0.35
20—25	2 692	15	0.56	3.15
25—30	2 407	14	0.58	6.05
30—35	1 635	15	0.91	10.60
35—40	819	7	0.85	14.85
40—45	266	4	1.50	22.35
		Plural-born prematures 2		
15—20	736	—	—	0
20—25	721	9	1.25	6.25
25—30	617	8	1.30	12.75
30—35	404	2		16.45
35—40	197	3	0.74	20.15
40—45	73	—		23.85
		Single-born controls 3		
15—20	4 306	7	0.16	0.80
20—25	4 137	25	0.60	3.80
25—30	3 700	27	0.73	7.45
30—35	2 579	21	0.81	11.50
35—40	1 339	7	0.52	14.10
40—45	491	2	0.41	16.15

did not exhibit asocial behaviour to any greater extent than did the controls. Moreover, the cumulative risks for the single-born prematures of being sentenced for crimes are consistently lower than the mean figures for the male population in towns in Sweden (DAHLBERG 1948). The corresponding risk figures for the controls are almost identical with these mean figures.

It must, however, be pointed out that in series such as the present, the reliability of the figures decreases considerably after 35 years of age, owing to the relatively small number of the subjects observed after this age. This also applies to the cumulative risk of being recorded in the register of the National Liquor Control Board. The rapid increase in the figures for the cumulative risk for both the single-born and the plural-born prematures cannot be accorded any great importance, since the number of years of observation decreases sharply for the 5-year periods after 35 years of age.

CHAPTER X

General Summary

It is evident from the review of the literature (*Chapter II*) that opinions regarding the mental and physical prognosis for prematurely born children are at variance, as are the figures obtained in the different investigations. This is presumably due mainly to the differences in the respective series with regard to such factors as the social status and post-natal care and to the fact that the series were often small and comparisons were seldom made with comparable control series.

The present series of prematures consists of 999 boys with a birth weight of 2500 grams or less, born at the three largest maternity hospitals in Stockholm between 1902 and 1921. They were all born in wedlock and were discharged alive from hospital. The control series consists of 1002 so-called social twins with a birth weight between 2 760 and 3 750 grams (Tables 1–3).

Comparisons are, as a rule, made between the following three series:

1. 759 premature single-born children
2. 240 premature plural-born children
3. 981 single-born controls.

See Tables 4–6.

An account of the obstetric, paediatric and social conditions is given in *Chapter IV* (Tables 7—17). On the whole it confirms already established facts. but also serves as a basis for the subsequent steps in the investigation. A summary of these conditions is recorded in Table 18.

The mortality among those children discharged alive from the maternity hospitals is reported in *Chapter V* (Tables 19—27). The results may be summarized as follows:

A. As could be expected, the mortality rate is higher for the prematurely born children than for the controls, even though all the children were discharged alive from hospital. The difference in the mortality is greatest during the first months of life and does not become evened out until 2—3 years of age (Tables 25 and 26). The difference is caused mainly by a considerable excess mortality from infectious diseases among the prematurely born children (Table 20). The mortality is highest among those in the lowest birth weight group (Table 22), those in the lowest social group (Table 23), those whose mothers were ill during pregnancy (Table 24) and those with several older siblings (Table 27).

B. A comparison between the single-born and the plural-born prematures discloses the surprising fact that the mortality is consistently higher for the plural-born children. This difference between the two series of prematurely born children is statistically probable, both for the total mortality and for the mortality from infectious diseases (Table 20). In social group III, the difference is statistically significant (Table 22). The higher mortality rate for the plural-born premature children as compared to the single-born prematures applies when the birth weight is the same in both categories (Table 22) when the condition of the mother during pregnancy is the same (Table 24) and with the same birth order number (Table 27).

An account is given in *Chapter VII* of certain physical and mental deviations from the norm, *i. e.*, mental developmental disturbances, diseases resulting in mental or physical disablement and tuberculosis. Only the percentage figures are given here, as well as the differences between the single-born prematures (1) and the single-born controls (3).

A. *Mental developmental disturbances*

	1	2 Per cent	3
a. Pupils in Special Classes (Table 29)	3.3	5.8	1.7
Difference 1–3: not significant.			
b. Institutional care (Table 30)	4.8	2.1	1.2
Difference 1–3: statistically significant.			
c. Pensions: spastic paralysis, epilepsy, educable and ineducable mental deficiency (Table 31)	3.5	2.8	0.7
Difference 1–3: statistically significant.			
n. b. the same individual is frequently recorded under a, b and c.			

B. *Disabling physical diseases*

	1	2 Per cent	3
Other illnesses than those under A entitling to a pension (Table 31)	3.1	0	3.0
Difference 1–3: not significant.			

C. *Tuberculosis*

	1	2 Per cent	3
a. Morbidity (Table 32)	11.0	14.6	13.0
Difference 1–3: not significant.			
b. Mortality after 1 year of age (Table 32)	3.6	2.8	3.8
Difference 1–3: not significant.			

The physical development at adult age and the fitness for military service are evaluated on the basis of the notations in the military registers (*Chapter VIII*).

	1	2	3
		Per cent	
a. Fully fit for active service (Table 36)	56.2	62.6	63.8
Difference 1–3: not significant.			
b. Unfit for active service (Table 36)	17.1	15.3	12.5
Difference 1–3: not significant.			
c. Military promotion of those fully fit	15.0	4.9	12.3
Difference 1–3: not significant.			
d. Mean height at 20 years of age (Table 33)	172.8 cm.	172.9 cm.	175.6 cm.
Difference 1–3: statistically significant.			
e. Mean body weight at 20 years of age (Table 34)	64.0 kg.	65.0 kg.	67.0 kg.
Difference 1–3: statistically significant.			

The social and economic prognosis is assessed from several aspects (*Chapter IX*). In the following table, the majority of the data are given as the percentages.

	1	2	3
		Per cent	
a. The social group of the subject (Table 38)			
Social group I	10.1	10.8	12.4
Social group III	45.6	50.0	43.6
b. Various forms of public relief (Table 39)			
Unemployment relief	16.9	21.3	14.6
Poor relief	20.4	22.3	21.8
Sickness relief	10.2	10.7	12.1
Other relief	17.1	23.3	18.1

Here as well, it must be recalled that the same individual may have received more than one form of relief.

	1	2	3
c. Median for net income in Swedish crowns (Table 41) . .	6080	5900	6270
d. Convictions for crime (Table 42) per cent	5.8	3.5	8.5
Convictions for drunkenness (Table 44) per cent . . .	11.0	15.3	10.9

There are no statistically significant differences between 1 (premature single-born children) and 3 (single-born controls) with regard to any of the social and economic conditions listed in the aforegoing.

Conclusions

Thus, a comparison is made between a relatively uniform series of prematurely born children, whose initial social status was not markedly poor, and a control series of children for whom the conditions were similar to that of the prematures in most respects, but whose birth weight was normal. Such a comparison permits the following conclusions to be drawn, at any rate with regard to the present series:

1. The mortality rate is considerably higher among the prematurely born children during the first two years of life, particularly among the plural-born prematures.

2. Among those prematurely born children who survive the first two to three years of life, there is a moderately higher incidence of such disorders that are usually considered to be associated with birth injuries than among the controls. The difference is statistically significant. In other respects, and with regard to other disabling diseases and tuberculosis, there is no difference between the prematurely born children and the controls.

3. There is a statistically significant difference between the height and body weight at 20 years of age in the two categories, the figures being lower for those prematurely born than for the controls. The incidence of those able to do their military service is practically the same in both categories and those prematurely born performed their military duties, on the whole, as well as did the controls.

4. As regards social adaptation, there are no statistically significant or probable differences between the prematurely born children and the controls. The general impression is that under no conditions were they more of a burden on the community than were the controls and that, in the majority of respects, they did as well as the controls.

Résumé

Ainsi qu'il ressort du *Chapitre II* dans lequel fut passée en revue la littérature, les pronostics sur l'évolution mentale et physique des enfants prématurés présentent de grandes variations, de même que font les résultats obtenus au moyen des différentes enquêtes. Ceci peut être attribué aux différences des séries respectives, particulièrement en ce qui concerne certains facteurs tels que la situation sociale et les soins post-natals. De plus, les séries étaient souvent réduites et il fut rarement procédé à des comparaisons entre elles et des séries de cas-témoins.

La série suivante d'enfants prématurés consiste de 999 garçons nés dans les trois plus grandes maternités de Stockholm, entre les années 1902—1921. Leur poids à la naissance était de 2.500 grammes et moins. Les cas considérés

sont uniquement des enfants légitimes, ayant quitté la maternité vivants. La série de cas-témoins consiste de 1.002 «jumeaux sociaux», ayant à la naissance un poids entre 2.760 et 3.750 grammes (Tab. 1—3).

En principe, les comparaisons sont faites entre les trois séries suivantes:

1. 759 enfants prématurés, naissances simples,
2. 240 enfants prématurés, naissances multiples,
3. 981 cas-témoins, naissances simples.

Voir Tables 4–6.

Dans le *Chapitre IV* (Tab. 7–17) est donné un aperçu sur les conditions obstétriques, pédiatriques et sociales concernant les enfants. En gros, il ne fait que confirmer un certain nombre de faits déjà établis. Cependant, il peut être utilisé comme base aux enquêtes ultérieures. On trouvera un résumé de ces conditions dans le tableau 18.

Au *Chapitre V*, on trouvera un aperçu sur la mortalité enregistrée parmi les enfants ayant quitté vivants la maternité (Tab. 19–27). Les résultats peuvent être résumés ainsi qu'il suit:

A. Ainsi que l'on pouvait s'y attendre, le taux de mortalité parmi les enfants prématurés est plus élevé que celui enregistré parmi les cas-témoins. La différence entre ces deux taux de mortalité est surtout sensible au cours des premiers mois, et ne s'aplanit qu'à l'âge de 2 à 3 ans (Tab. 25 et 26). Cette différence est impliquable principalement à la mortalité occasionnée chez les enfants prématurés par les maladies infectieuses (Tab. 20). On note les taux de mortalité les plus élevés chez: les enfants dont le poids à la naissance est le moindre (Tab. 22), les enfants originaires du groupe social le plus bas (Tab. 23), les enfants dont la mère fut malade au cours de sa grossesse (Tab. 24) et enfin les enfants ayant plusieurs frères ou sœurs plus âgés (Tab. 27).

B. Une comparaison entre la série de prématurés, naissances simples, et celle de prématurés, naissances multiples, fait ressortir ce fait surprenant: le taux de mortalité est sensiblement plus élevé chez les enfants nés jumeaux ou multiples que chez les enfants nés seuls. Cette différence entre les deux séries d'enfants prématurés est statistiquement probable, et pour le taux de mortalité totale, et pour le taux de mortalité due à des maladies infectieuses (Tab. 20). La différence a une signification statistique dans le groupe social III (Tab. 22). Même quand le poids à la naissance est le même pour les sujets des deux séries d'enfants prématurés, la différence entre les deux taux de mortalité apparaît (Tab. 22). Il en va de même quand la condition de la mère est identique pendant la grossesse (Tab. 24), et le numéro d'ordre de naissance également identique (Tab. 27).

Dans le *Chapitre VII* est donné un aperçu sur quelques anormités physiques et mentales, telles que l'arriération mentale, maladies résultant en invalidité

physique ou mentale et la tuberculose. Seules les donnés procentuelles sont indiquées, de même que les différences entre les enfants prématurés, naissances simples (1) et les cas-témoins, naissances simples (3).

A. *Troubles du développement mental*

	1	2	3
		%	
a. Elèves en classes spéciales (Tab. 29)	3.3	5.8	1.7
Différence 1—3: sans signific.			
b. Soins en établissements spéciaux (Tab. 30)	4.8	2.1	1.2
Différence 1—3: statistiq. signif.			
c. Pensions: paralysie spasmodique, épilepsie, arriération mentale éducable et inéducable (Tab. 31) . . .	3.5	2.8	0.7
Différence 1—3: statistiq. signif.			

n. b. le même sujet est souvent enregistré sous a, b et c.

B. *Maladies physiques causes d'incapacités*

	1	2	3
		%	
Maladies autres que celles déjà enregistrées sous A et donnant droit à une pension (Tab. 31)	3.1	0	3.0
Différence 1—3: sans signific.			

C. *Tuberculose*

	1	2	3
		%	
a. Morbidité (Tab. 32)	11.0	14.6	13.0
Différence 1—3: sans signific.			
b. Mortalité après l'âge de 1 an (Tab. 32)	3.6	2.8	3.8
Différence 1—3: sans signific.			

Le développement physique des sujets à l'âge adulte et leur aptitude au service militaire sont donnés en fonction des consignations enregistrées sur les registres militaires (*Chapitre VIII*).

	1	2	3
		%	
a. Aptes au service armé (Tab. 36) .	56.2	62.6	63.8
Différence 1—3: sans signific.			
b. Inaptes au service armé (Tab. 36)	17.1	15.3	12.5
Différence 1—3: sans signific.			

	1	2	3
		%	
c. Avancement en grade des aptes au service armé	15.0	4.9	12.3
Différence 1—2: sans signific.			
d. Taille moyenne à l'âge de 20 ans (Tab. 33)	172.8 cm	172.9 cm	175.6 cm
Différence 1—3: statistiq. signific.			
e. Poids moyen à l'âge de 20 ans (Tab. 34)	64.0 kg	65.0 kg	67.0 kg
Différence 1—3: statistiq. signific.			

Les pronostics sociaux et économiques sont faits en considération de plusieurs facteurs (*Chapitre IX*). La majorité des données des tables suivantes sont procentuelles.

	1	2	3
		%	
a. Groupe social du sujet (Tab. 38)			
Groupe social I	10.1	10.8	12.4
Groupe social III	45.6	50.0	43.6
b. Formes de secours publique (Tab. 39)			
Alloc. chomage	16.9	21.3	14.6
Assistance publique	20.4	22.3	21.8
Alloc. maladie	10.2	10.7	12.1
Secours divers	17.1	23.3	18.1

Ici encore, il importe de souligner que le même sujet peut avoir reçu des secours sous plusieurs formes.

	1	2	3
c. Revenu moyen net en Cour. Suéd. (Tab. 41)	6080	5900	6270
d. Condamnations pour crimes (Tab. 42), en %	5.8	3.5	8.5
e. Condamnations pour alcoolisme (Tab. 44), en %. . .	11.0	15.3	10.9

Entre 1 (enfants prématurés, naissances simples) et 3 (cas-témoins, naissances simples) il n'existe pas de différences statistiquement significatives, si l'on considère les conditions sociales et économiques enregistrées précédemment.

Conclusions

Ainsi, une comparaison a été faite entre une série rélativement uniforme d'enfants prématurés, ayant une condition sociale de base qui n'est pas caractérisable comme particulièrement pauvre, et une série de cas-témoins, dont les conditions ressemblaient à celles des précédents, mais qui avaient un poids normal à la naissance. De cette comparaison, on peut tirer les conclusions suivantes, en tout cas en ce qui concerne la série en question ici:

1. Le taux de mortalité est considérablement plus élevé au cours des deux premières années chez les enfants prématurés et tout spécialement dans les cas de naissances multiples.

2. Certaines maladies généralement considérées comme étant la conséquence d'accidents lors de la naissance se rencontrent plus fréquemment parmi les enfants prématurés qui survivent l'âge de deux ou trois ans, que parmi les cas-témoins. Cette différence a une signification statistique. Dans les autres domaines, l'on ne recontre aucune différence entre les enfants prématurés et les cas-témoins, ni quant aux maladies causes d'invalidités et la tuberculose.

3. L'on enregistre une différence statistiquement significative entre les tailles et les poids des deux catégories de sujets ayant atteint l'âge de 20 ans. Les chiffres concernant les enfants prématurés sont inférieurs à ceux concernant les cas-témoins. Le nombre des sujets classés aptes au service armé est sensiblement le même dans les deux catégories de sujets. En somme, les prématurés accomplirent leur service militaire d'une manière aussi satisfaisante que les cas-témoins.

4. En ce qui concerne l'adaptation au milieu social, il n'existe pas de différence ayant une signification statistique ou probable, entre les enfants prématurés et les cas-témoins. Il semble que l'on puisse affirmer que les enfants prématurés ne furent en aucun cas un fardeau spécial pour la société. On peut dire, en effet, que dans la majorité des aspects ils réussirent leur vie aussi bien que les cas-témoins.

Zusammenfassung

Aus der Literaturübersicht geht hervor, dass die Meinungen über die mentale und physische Prognose bei prämatur geborenen Kindern verschieden sind. Dieses beruht offenbar auf Verschiedenheiten zwischen den Materialen in Hinblick auf die sozialen Verhältnisse, Pflegeverhältnisse etc., sowie auf der Tatsache, dass das Material oft klein war und das selten mit vergleichbarem Kontrollmaterial verglichen wurde. Die in dieser Untersuchung angewandten Fälle sind deshalb auf bestimmte Weise ausgewählt worden: Das Material besteht aus 999 prämatur geborenen Knaben mit einem Geburtsgewicht von $\leqq 2500$ g., die in den Jahren 1902—1921 in drei Stockholmer

Entbindungsanstalten geboren wurden. Es handelt sich um eheliche Kinder, die lebend aus der Entbindungsstation entlassen wurden. Als Kontrollmaterial dienen 1002 Kinder, sogenannte soziale Zwillinge, deren Geburtsgewicht innerhalb normaler Grenzen liegt. Siehe Tabelle 1—3.

In dieser Untersuchung sind im allgemeinen die folgenden 3 Gruppen miteinander verglichen:

1. 759 prämature Einzelgeborene.
2. 240 prämature Mehrlingsgeborene.
3. 981 Einzelgeborene aus dem Kontrollmaterial.

Siehe Tabelle 4—6.

In *Kapitel IV* (Tabelle 7—17) wird über die geburtshilflichen, pädiatrischen und sozialen Bedingungen in diesen Gruppen berichtet. In dieser Darstellung werden hauptsächlich bereits bekannte Tatsachen bestätigt, aber sie dient auch als Basis in der folgenden Untersuchung. In Tabelle 18 sind diese Bedingungen zusammengefasst.

Über die Mortalität der lebend aus den Entbindungsanstalten entlassenen Kindern wird in *Kapitel V* Tabelle 19—27 berichtet. Das Resultat kann folgendermassen zusammengefasst werden.

A. Wie zu erwarten war ist die Sterblichkeit in der Gruppe der Frühgeborenen grösser als im Kontrollmaterial, obgleich es sich ja nur um Kinder handelt, die lebend aus der Entbindungsanstalt entlassen wurden. Der Unterschied in der Mortalität ist in den ersten Monaten am grössten und ist nicht vor einem Alter von 2—3 Jahren ausgeglichen (Tabelle 25 und 26). Der Unterschied beruht im Wesentlichen auf der erhöhten Frühgeburtensterblichkeit an Infektionskrankheiten (Tabelle 20), und ist am grössten in den niedrigsten Gewichtsklassen (Tabelle 22), den niedrigsten sozialen Schichten (Tabelle 23), sowie für Kinder kranker Mütter (Tabelle 24) und für Kinder mit mehreren Geschwistern (Tabelle 27).

B. Beim Vergleich von frühgeborenen Einlingen und Mehrlingen zeigt sich die überraschende Tatsache, dass Mehrlinge eine höhere Mortalität aufweisen. Diese Differenz zwischen den Prämaturengruppen ist sowohl im Hinblick auf die Totalsterblichkeit als auch im Hinblick auf die Sterblichkeit an Infektionskrankheiten statistisch wahrscheinlich (Tabelle 20). In der Sozialgruppe III ist der Unterschied gesichert (Tabelle 22). Die höhere Sterblichkeit der frühgeborenen Mehrlinge gegenüber den frühgeborenen Einlingen besteht bei gleichem Geburtsgewicht (Tabelle 22), bei gleichartigem Gesundheitszustand der Mutter (Tabelle 24), sowie bei gleicher Zahl in der Geschwisterreihe (Tabelle 27).

Im *Kapitel VII* wird über gewisse geistige und körperliche Abweichungen von der Norm berichtet. Es handelt sich um psychische Entwicklungs-

störungen, hochgradig invalidisierende seelische und körperliche Erkrankungen, sowie Tuberkulose. In diesem summarischen Bericht sind nur Prozentzahlen angegeben. Die Unterschiede werden nur zwischen prämaturen Einlingen (1) und Einzelgeborenen im Kontrollmaterial (3) angegeben.

A. *Mentale Entwicklungsstörungen*

	1	2	3
		%	
a. Hilfsschüler, Tab. 29	3.3	5.8	1.7
Differenz 1–3 nicht signifikant.			
b. Anstaltspflege. Tabelle 30	4.8	2.1	1.2
Differenz 1–3 signifikant.			
c. Pension. (Spastiker, Epileptiker und erziehbare und unerziehbare Geistesschwache) Tabelle 31	3.5	2.8	0.7
Differenz 1–3 signifikant.			

Es muss erwähnt werden, dass dieselbe Person oft in Gruppe a., b. und c. einbegriffen ist.

B. *Invalidisierende körperliche Erkrankungen*

	1	2	3
		%	
Alle ausser den unter A. angegebenen Erkrankungen, die pensionspflichtig sind. Tabelle 31.	3.1	0	3.0
Differenz 1–3 nicht signifikant.			

C. *Tuberkulose*

	1	2	3
		%	
a. Morbidität. Tabelle 32.	11.0	14.6	13.0
Differenz 1–3 nicht signifikant.			
b. Mortalität nach dem 1. Lebensjahr. Tabelle 32. . . .	3.6	2.8	3.8
Differenz 1–3 nicht signifikant.			

Die körperliche Entwicklung im Erwachsenenalter, sowie die Wehrtauglichkeit wurde aus Militärregistern ersehen. *Kapitel VIII.*

	1	2	3
		%	
a. Voll wehrtauglich, Tabelle 36 . .	56.2	62.6	63.8
Differenz 1–3 nicht signifikant.			
b. Wehruntauglich, Tabelle 36 . .	17.1	15.3	12.5

c. Militärische Beförderung von voll Wehrtauglichen	15.0	4.9	12.3
Differenz 1–3 nicht signifikant.			
d. Mittellänge mit 20 Jahren (Tabelle 33)	172.8 cm	172.9 cm	175.6 cm
Differenz 1–3 signifikant.			
e. Mittelgewicht mit 20 Jahren. Tab. 34	64.0 kg	65.0 kg	67.0 kg
Differenz 1–3 signifikant.			

Die soziale und ekonomische Prognose wurde unter verschiedenen Gesichtspunkten in *Kapitel IX* beurteilt. Die folgende Tabelle gibt die Angaben in Prozent wieder.

	1	2 %	3
a. Die eigene Sozialgruppe des Nachuntersuchten. Tabelle 38.			
Sozialgruppe I.	10.1	10.8	12.4
Sozialgruppe III.	45.6	50.0	43.6
b. Verschiedene Formen von Unterstützung. Tabelle 39			
Arbeitslosenunterstützung	16.9	21.3	14.6
Armenunterstützung	20.4	22.3	21.8
Unterstützung bei Krankheit	10.2	10.7	12.1
Übrige Unterstützungen	17.1	23.3	18.1
Auch hier muss erwähnt werden, dass die gleiche Person verschiedene Unterstützungen haben kann.			
c. Mittleres Einkommen in schwedischen Kronen. Tabelle 41	6080	5900	6270
d. Für Verbrechen gestraft. %. Tab. 42	5.8	3.5	8.5
Für Trunkenheit gestraft. %. Tabelle 44	11.0	15.3	10.9

Wie aus der oben stehenden Tabelle hervorgeht, besteht zwischen 1 (prämature Einzelgeborene) und 3 (einzelgeborene Kontrollen) weder in sozialer noch ekonomischer Hinsicht ein bedeutender statistischer Unterschied.

Wenn man ein so weit möglich homogen ausgewähltes Material von Prämaturen mit nicht allzu schlechter sozialer Ausgangslage mit einem in meister Hinsicht gleichartigen Kontrollmaterial mit normalen Geburtsgewicht vergleicht, so lässt sich zusammenfassungsweise folgendes sagen:

1. Die Mortalitätsziffer ist unter den frühgeborenen Kindern innerhalb der ersten beiden Lebensjahre beträchtlich höher und ist besonders bei den prämaturen Mehrlingen ausgesprochen.

2. Diejenigen, die die ersten 2–3 Jahre überleben, zeigen bei der Nachuntersuchung eine mässige, aber statistisch sichergestellte höhere Frequenz von Leiden, die man mit Geburtsschäden in Verbindung zu setzen pflegt. Was die invalidisierenden Krankheiten oder Tuberkulose betrifft, so besteht zwischen Prämaturen und Ausgetragenen kein Unterschied.

3. Die prämatur Geborenen haben noch mit 20 Jahren geringere Körperlänge und geringeres Gewicht als die Kontrollpersonen, was statistisch sichergestellt ist. Sie leisten ihre Wehrpflicht im grossen Ganzen gesehen ebensogut wie die Kontrollpersonen mit normalem Geburtsgewicht.

4. In sozialer Hinsicht bestehen keine statistisch bestätigten oder wahrscheinliche Unterschiede, und als Gesamteindruck kann man sagen, dass die prämatur geborenen Personen keineswegs dem Staat mehr zur Last fallen als die Personen mit normalem Geburtsgewicht, und dass sie sich in fast jeder Hinsicht ebenso gut durchsetzen.

Sumario general

De la revisión de la bibliografía se deduce que las opiniones sobre los niños nacidos con prematuridad varian por lo que se refiere al pronóstico mental y físico y así son los valores obtenidos en diferentes investigaciones. Esto es debido probablemente ante todo a las diferencias que existen en las respectivas series con respecto a aquellos factores como el estado social y cuidado postnatal y al hecho de que las series amenudo eran pequeñas y las comparaciones fueron raramente hechas con individuos de control comparables.

La presente serie de prematuros consiste de 999 niños con un peso de nacimiento de 2500 gramos o menos, nacidos en los tres hospitales maternales más grandes de Estocolmo entre 1902 y 1921. Estos nacieron todos en matrimonio y salieron del hospital en vida. Las series de control consisten en 1002 individuos llamados "gemelos sociales" con un peso de nacimiento alrededor de los valores normales (cuadros 1—3).

Las comparaciones fueron hechas, como regla, entre las siguientes tres series:

1. 759 niños prematuros nacidos en número único
2. 240 niños prematuros nacidos en número plural
3. 981 individuos de control nacidos en número único.

Véase cuadros 4—6.

En el *capítulo IV* (cuadros 7—17) se da cuenta de las condiciones obstétricas, pediatricas y sociales. En general se confirman hechos ya establecidos, pero que sirven de base para la subsiguiente investigación. Un sumario de estas condiciones se encuentra en el cuadro 18.

La mortalidad entre los niños que salieron del hospital maternal en vida se indican en el *capítulo V* (cuadros 19—27). Los resultados son en resumen los siguientes:

A. Como era de esperar la mortalidad es más elevada en los niños nacidos con prematuridad que en los individuos de control, a pesar de que todos los niños salieron con vida del hospital. La diferencia en la mortalidad alcanza su punto culminante durante los primeros meses de la vida y no se situa al mismo nivel hasta los dos a tres años de edad (cuadros 25 y 26). La diferencia tiene su causa principal en la alta mortalidad debida a enfermedades infecciosas entre los prematuros (cuadro 20). La mortalidad más elevada se encuentra entre aquellos niños nacidos con el peso más bajo (cuadro 22), entre los nacidos en el grupo social más bajo (cuadro 23), entre aquellos cuyas madres sufrieron enfermedades durante el embarazo (cuadro 24) y entre aquellos con varios hermanos mayores (cuadro 27).

B. Una comparación entre los niños que nacieron en número único o plural nos revela el hecho sorprendente de que la mortalidad es considerablemente mayor entre los niños nacidos en número plural. Esta diferencia entre las dos series de niños nacidos con prematuridad es estadisticamente probable, tanto por lo que respecta la mortalidad total como la mortalidad debida a enfermedades infecciosas (cuadro 20). En el grupo social III, la diferencia es de importancia estadistica (cuadro 22). El alto número de mortalidad entre los niños nacidos en número plural en comparación con los prematuros nacidos en número único se evalua si el peso de nacimiento es el mismo en las dos categorias (cuadro 22), si las condiciones de la madre durante el embarazo son las mismas (cuadro 24) y si el número de órden de nacimiento es el mismo (cuadro 27).

En el *capítulo VII* se da cuenta sobre ciertas deviaciones de la normalidad físicas y mentales, a saber, alteraciones del desarrollo mental, enfermedades que provienen de impedimientos mentales y físicos y tuberculosis. Aquí solamente se dan a conocer los porcentajes así como las diferencias entre los niños prematuros nacidos en número único (1) y los individuos de control (3) también nacidos en número único.

A. *Perturbaciones del desarrollo mental*

	1	2	3
	Por ciento		
a. Discípulos en clases especiales (cuadro 29)	3.3	5.8	1.7
Diferencia 1—3: sin importancia			
b. Al cuidado de instituciones (cuadro 30)	4.8	2.1	1.2
Diferencia 1—3: de importancia estadística			

	1	2	3
		Por ciento	
c. Pensiones: parálisis espástica, epilepsia, deficiencia mental educable y no educable (cuadro 31).	3.5	2.8	0.7
Diferencia 1—3: de importancia estadística.			

adv.: el mismo individuo se registra amenudo entre a, b y c.

B. *Enfermedades de imposibilidad física*

	1	2	3
		Por ciento	
Otras enfermedades que las que se citan bajo con título pensiones (cuadro 31)	3.1	0	3.0
Diferencia 1—3: sin importancia.			

C. *Tuberculosis*

	1	2	3
		Por ciento	
a. Morbididad (cuadro 32)	11.0	14.6	13.0
Diferencia 1—3: sin importancia.			
b. Mortalidad después del 1er año de edad (cuadro 32).	3.6	2.8	3.8
Diferencia 1—3: sin importancia.			

El desarrollo físico en la edad adulta y la aptitud para el servicio militar han sido evaluados con la ayuda de las anotaciones de los registros militares (*capítulo VIII*).

	1	2	3
		Por ciento	
a. Aptitud completa para el servicio activo (cuadro 36)	65.2	62.6	63.8
Diferencia 1—3: sin importancia.			
b. No aptos para el servicio activo (cuadro 36)	17.1	15.3	12.5
Diferencia 1—3: sin importancia.			
c. Promoción militar de aquellos completamente aptos	15.0	4.9	12.3
Diferencia 1—3: sin importancia.			
d. Altura media a los 20 años de edad (cuadro 33)	172.8 cm	172.9 cm	175.6 cm
Diferencia 1—3: de importancia estadística.			
e. Peso medio a los 20 años de edad (cuadro 34)	64.0 kg	65.0 kg	67.0 kg
Diferencia 1—3: de importancia estadística.			

El pronóstico social y económico se evalua de diferentes aspectos (*capítulo IX*). En el cuadro siguiente la mayoría de los datos se dan en el tanto por ciento.

	1	2	3
		Por ciento	
a. Grupo social del individuo (cuadro 38)			
Grupo social I	10.1	10.8	12.4
Grupo social III	45.6	50.0	43.6
b. Formas distintas de socorro público (cuadro 39)			
Socorro sin empleo	16.9	21.3	14.6
Socorro escaso	20.4	22.3	21.8
Socorro de enfermedad	10.2	10.7	12.1
Otras clases de socorro	17.1	23.3	18.1

Hay que recordar aqui que el mismo individuo puede haber recibido más de una clase de socorro.

	1	2	3
		Por ciento	
c. Ingreso medio limpio en coronas suecas (cuadro 41)	6080	5900	6270
d. Pruebas de criminalidad por ciento (cuadro 42)	5.8	3.5	8.5
e. Pruebas de embriaguez por ciento (cuadro 44)	11.0	15.3	10.9

No existen diferencias de importancia estadística entre 1 (niños prematuros nacidos en número único) y 3 (individuos de control nacidos en número único) por lo que respecta a cualquiera de las condiciones sociales y económicas anotadas en lo antedicho.

Conclusiones

De este modo, se hace una comparación entre series relativamente uniformes de niños prematuros, cuyo estado social inicial no era marcadamente pobre y series de control de niños para los cuales las condiciones fueron parecidas a aquellos de los prematuros en la mayoría de los aspectos, pero cuyo peso de nacimiento era normal. Una tal comparación permite sacar las conclusiones siguientes:

1. La mortalidad es considerablemente más alta entre los niños nacidos con prematuridad durante los dos primeros años de vida especialmente entre los prematuros nacidos en número plural.

2. Entre aquellos niños nacidos prematuramente que sobrevivieron de los dos a los tres años de vida, hay una incidencia moderadamente más alta de aquellas perurbaciones que generalmente se consideran estar asociadas a daños de parto que entre los individuos de control. La diferencia tiene importancia

estadística. En otros aspectos y por lo que se refiere a otras enfermedades de incapacidad y tuberculosis, no hay diferencia entre los niños nacidos con prematuridad y los individuos de control.

3. Hay una diferencia de importancia estadística entre la altura y el peso a los 20 años de edad en las dos categorías, siendo los números más bajos para aquellos nacidos prematuramente que para los individuos de control. La incidencia de los que fueron capaces prestar su servicio militar es prácticamente la misma en ambas categorías y los nacidos con prematuridad cumplieron su deber militar por lo general tan bien como lo hicieron los individuos de control.

4. Por lo que se refiere a la adaptación social, no existen diferencias de importancia estadística o probables entre los niños nacidos prematuramente y los individuos de control. La impresión general es de que bajo ninguna condición suponieron una carga mayor para la comunidad de lo que hicieron los individuos de control y de que en la mayoría de los aspectos lo fueron tanto como aquellos.

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